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EXTRACTION OF ADSORBED CATIONS FROM SOIL BY ELECTRODIALYSIS

BENJAMIN D. WILSON

Cornell University

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Cations are extracted differentially from soil by electrodialysis. Mattson (6) found, by means of fractional electrodialysis, that calcium was removed with relatively greater ease from the colloidal material extracted from a soil than was magnesium, iron, or aluminum. In like manner, but using a different type of electrical cell, the writer (8) observed this same cationic relationship to hold with respect to a clay soil. In each of these experiments the material which was subjected to electrodialysis contained all of the cations which were characteristic of it. Of these calcium predominated.

The experiments of the present investigation differ from those just cited in that they are concerned with the ease or rapidity with which certain cations appear in the diffusates of electrodialyzed soil when it is saturated with respect to a single cation or a particular combination of cations.

DESCRIPTION OF SOIL

A soil designated as Vergennes clay was employed in the investigation. It is a productive soil derived from the decomposition of glacial-lake clay.[1] It contains approximately 12 mgm. equivalents of exchangeable cations to 100 gm. of air-dry soil as determined by electrodialysis. It has a reaction of pH 5.4 and is classified as a soil of good fertility. In preparation for analysis the soil was air-dried and passed through a-1 mm. sieve. Stones larger than 1 mm. were discarded.

METHODS EMPLOYED

Ten grams of soil were elctrodialyzed by the use of a two-compartment-Pyrex cell (8) similar in design to the one devised by Bradfield (1). Electrodialysis was conducted for 8 hours under the influence of a direct current of 110 volts, which was regulated by means of a sliding-contact rheostat. During this period about one liter of diffusate was obtained. The amperage varied during the process of dialysis; it was seldom higher than 100 milliamperes; at the close of the process it was occasionally as low as 5 milliamperes.

[1] The soil contains 4.3 per cent, 1.4 per cent, and 3.1 per cent respectively of calcium, magnesium, and potassium.

After being subjected to electrodialysis, the soil was freed of soluble anions by means of distilled water and suction while in the alundum thimble in which electrodialysis occurred. At this stage the alumino-silicic complex of the soil was more or less saturated with hydrogen containing few or no other exchangeable cations, and the reaction of the soil approached pH 4.0. The soil was then air dried and transferred to a small beaker. Any soil adhering to the walls of the thimble was loosened and washed into the beaker with a part of the solution containing the cation or cations with which the soil was to be treated. In most instances this was a normal solution of a salt of acetic acid or a combination of several salts of acetic acid in molecular equivalent quantities. One hundred cubic centimeters of solution was added to the soil and the mixture frequently stirred. The soil was allowed to settle for 24 hours and the supernatant liquid was then withdrawn with a pipette. This procedure was repeated successively with two fresh portions of solution, after which the soil was washed several times with distilled water by decantation and transferred to an alundum thimble. The washing was continued, suction being used, until the filtrate gave no test for the ions with which the soil had been treated. When air dry, the soil was brought into suspension with a small quantity of distilled water and electrodialyzed in the manner already described to remove the particular cation or cations which it had adsorbed.[2]

The total quantity of cations in the diffusates was determined by titration with suitable reagents (8). Individual cations were ascertained by standard methods of chemical analysis. Hydrogen-ion concentrations were determined potentiometrically, a quinhydrone electrode being used.

CATIONS EXTRACTED BY ELECTRODIALYSIS FROM SOIL SATURATED WITH HYDROGEN BEFORE TREATMENT

The cations present in the diffusates of the electrodialyzed soils, after being subjected to the several treatments outlined in the foregoing, are recorded in table 1. Normal solutions of potassium, calcium, and magnesium acetates were employed. The solution of aluminum acetate was approximately 0.6 N, its being the limit of solubility in distilled water of the salt at hand. As indicated in the table, electrodialysis was conducted fractionally. The first fraction (A) represents the cations which were extracted from the soil during a period of 2 hours; the second (B) and the third fractions (C) each represent the quantity extracted during a period of 3 hours. The total quantity of cations in each fractional diffusate, determined by titration and expressed in terms of NaOH, may be compared with its content of the cation with which the soil was treated. Such a comparison shows that virtually the only cation present in the diffusates was the one which was introduced into the soil.

Practically equivalent quantities of cations were extracted from the soils treated with potassium or calcium acetate. But potassium was removed from

[2] The word "adsorption," as used in this paper, is not intended to distinguish between physical and chemical forces.

the soil with greater rapidity than was calcium. This may be seen by comparing the quantities of the cations present in the diffusates. Five hours were required to extract as much calcium from the soil as was extracted in two hours with respect to potassium. Magnesium was removed with comparative difficulty from the soil treated with mangesium acetate. The quanitity extracted in 8 hours was approximately 50 per cent of the quantity of potassium or calcium removed during a period of equal length. Although the amount of magnesium was smaller in each successive fraction, its rate of decrease was exceedingly small as compared with that of potassium or calcium.

TABLE 1

Cations extracted from soil by fractional electrodialysis

(Soil electrodialyzed before treatment)

TREATMENT	FRACTION	MILLIGRAM EQUIVALENTS PER 100 GM. OF AIR-DRY SOIL	
		Cation of treatment	Total cations in terms of NaOH
Potassium acetate	A	17.8	18.6
	B	1.5	2.4
	C	0.2	0.4
	Total	19.5	21.4
Calcium acetate	A	12.1	12.5
	B	5.4	6.2
	C	1.1	1.4
	Total	18.6	20.1
Magnesium acetate	A	3.8	3.7
	B	2.8	3.0
	C	2.5	2.1
	Total	9.1	8.8
Aluminum acetate	A	0.2	0.3
	B	0.1	0.1
	C	0.1	0.1
	Total	0.4	0.5

Very little aluminum was found in the diffusates of the soil treated with aluminum acetate. Less than 1 mgm. equivalent was removed to the cathode chamber during 8 hours of electrodialysis. The limited quantity of magnesium and the exceedingly small quantity of aluminum extracted from the soil, as compared with the quantities of potassium and calcium extracted, raised the question as to the actual amounts of magnesium and aluminum adsorbed by the soil. If these latter cations were adsorbed to the same degree as were potassium and calcium, it is obvious that their behavior with respect to elec-

trodialysis differed fundamentally from that of the other two cations. This phase of the subject will be discussed presently.

With aluminum omitted from the discussion for the present, it may be said that the ease with which the following cations were removed by electrodialysis from the soil of the investigation, when only one of them was presented for adsorption, was in the descending order K > Ca > Mg. When they were adsorbed in the presence of one another the same order prevailed. This is shown in table 2. The data there recorded were obtained as a result of treating the soil with a normal solution of potassium, calcium, and magnesium acetates in molecular equivalent quantities. The method of treating the soil was precisely that which was employed when the soil was treated with only one of these cations. Potassium was removed with the greatest rapidity, followed by calcium and magnesium in the order named. A direct relationship is seen to exist between the ease of cationic extraction and the quantity of cations removed. Potassium, which was removed with the greatest rapidity, was also

TABLE 2

Cations extracted by fractional dialysis from soil treated with potassium, calcium, and magnesium in molecular equivalent quantities

(Soil electrodialyzed before treatment)

FRACTION	MILLIGRAM EQUIVALENTS PER 100 GM. OF AIR-DRY SOIL				
	K	Ca	Mg	Total	Total cations in terms of NaOH
A	8.1	3.3	0.8	12.2	11.8
B	1.4	3.6	1.8	6.8	6.0
C	0.5	0.4	1.7	2.6	1.8
Total.......	10.0	7.3	4.3	21.6	19.6

removed in largest amount. The converse was true for magnesium. The sum of the quantities of the three cations extracted from the soil in 8 hours was in stoichiometric agreement with the quantity of potassium or calcium removed during an equal period of time when the soil was treated with one or the other of these cations. This relationship would imply that most of the magnesium which was adsorbed in the presence of potassium and calcium was extracted in 8 hours. This was probably the case, for it would seem from the data presented that the adsorption of mangesium, under the conditions of the experiment, was relatively small. In that event, any magnesium that may have remained in the alumino-silicic complex of the soil after 8 hours of electrodialysis was not in sufficient quantity to affect materially the total amount of cations recovered during that time.

It is not to be assumed that complete saturation of the soil occurred with respect to any one of the treatments. It seems probable, however, in the light of the results shown in tables 1 and 2, that the soil was near the point of cationic

saturation after treatment with potassium or calcium acetate or a combination of these with magnesium acetate.

Cations extracted by electrodialysis from soil saturated with calcium before treatment

In the experiments just reviewed the alumino-silicic complex of the soil was saturated with hydrogen before the soil was treated with the cations of the acetate solutions. When the complex was in that condition, as has been shown, the total recovery of the added cations from the soil by means of electrodialysis was decidedly different. The difference in the recovery of potassium and calcium on the one hand, and magnesium and aluminum on the other, suggested that magnesium may react to replace only a fraction of the exchangeable hydrogen in the alumino-silicic complex of the soil and that aluminum may not cause the replacement of any of it. This contingency led to the following experiment which was designed to determine whether more of the

TABLE 3

Calcium removed from soils treated with calcium acetate by potassium, magnesium and aluminum acetates and the extraction of these latter cations by electrodialysis

(Soils electrodialyzed before treatment with calcium acetate [pH 7.6])

FINAL TREATMENT OF SOIL	MILLIGRAM EQUIVALENTS PER 100 GM. OF AIR-DRY SOIL		
	Ca removed by K, Mg, or Al acetate	K, Mg, or Al in diffusate	Total cations in diffusate in terms of NaOH
Potassium acetate (pH 7.7)	18.1	18.9	20.3
Magnesium acetate (pH 7.5)	18.2	7.1	9.0
Aluminum acetate (pH 3.5)	17.8	....	0.6

magnesium and aluminum added to the soil as acetates could be recovered by electrodialysis if the replaceable hydrogen of the soil was first replaced with calcium. That comparisons might be made between the action of magnesium and aluminum with that of potassium, tests using potassium acetate were conducted concurrently.

To this end soils were electrodialyzed and treated with calcium acetate. If a soil with its alumino-silicic complex saturated with calcium is treated with potassium, magnesium, or aluminum acetate and the supernatant liquid and wash water resulting from each of the treatments are analyzed for calcium, it should be possible to ascertain the extent to which each of these three cations will bring about the release of the adsorbed calcium. If the soil resulting from each of the three treatments is electrodialyzed, it should be possible to compare the degree of extractability of potassium, magnesium, and aluminum with the quantity of calcium released by each of the treatments.

The results which were obtained in following these two procedures are re-

corded in table 3. Each cation is seen to have liberated to the supernatant liquid approximately equal quantities of calcium. The values referred to are slightly less than the quantity of calcium that was obtained by electrodialyzing the soil when saturated with calcium acetate, as is shown in table 1. Nevertheless, the differences are within the limits of probability and it appears safe to conclude that each of the three cationic treatments released from the soil an equal quantity of absorbed calcium. But the quantities of cations recovered from the residual soil by electrodialysis do not indicate that, with the replacement of the adsorbed calcium, equal quantities of potassium, magnesium, and aluminum entered the alumino-silicic complex of the soil. Potassium, no doubt, did enter the adsorbing complex stoichiometrically in replacing calcium. But the results of table 3 do not show this relationship to obtain with respect to magnesium or aluminum. Aluminum was not determined in the diffusate of the soil treated with aluminum acetate. Its determination did not appear necessary because of the small total cationic content of the diffusate as determined by titration. This value is shown in terms of NaOH. It is not clear from the data presented whether magnesium and aluminum entered the soil complex as exchangeable ions and were not extractable by electrodialysis to the same extent as was potassium, or whether they were precipitated within the soil mass, bringing about the liberation of calcium by means of secondary reactions. Kelley and Brown (4) have expressed the view that cationic replacement in soil resulting from the addition of aluminum chloride is induced by the presence of hydrogen ions resulting from the hydrolysis of the aluminum salt and not by the aluminum ions directly.

Adsorption of calcium by electrodialzyed soils after treatment with magnesium or aluminum

In an attempt to determine the degree to which magnesium and aluminum entered the adsorbing complex of the electrodialyzed soil, it was treated first with magnesium or aluminum acetate and later with calcium acetate. It appeared to the writer that by analyzing the supernatant liquids resulting from the calcium-acetate treatments for magnesium and aluminum respectively, and by electrodialyzing the residual soils, some knowedge of the quantities of these cations which entered the adsorbing complex of the soil might be ascertained. The intent of this latter statement is apparent from table 4.

It will be seen that approximately 20 mgm. equivalents of magnesium was extracted from the soil when it was treated with calcium acetate. In the liberation of the magnesium an equal quantity of calcium was adsorbed by the soil. This is shown by the quantity of calcium which was present in the diffusate of the soil that had been treated with magnesium acetate when it was electrodialyzed. No other cation was present in the diffusate in appreciable amount, for its total cationic content was practically that of its calcium content. The quantity of magnesium shown to be liberated from the soil is equivalent to the quantity of calcium that was found to be adsorbed by the soil in the earlier ex-

periments. The data in table 4 which refer to magnesium, together with certain of those in tables 1 and 3, appear to support the conclusion that the soil of the investigation, after being subjected to electrodialysis, adsorbed equal quantities of potassium, calcium, and magnesium as acetates but that the latter cation was more difficult of extraction by electrodialysis than was either potassium or calcium.

Only a trace of aluminum was obtained from the soil previously treated with aluminum acetate when it was brought in contact with calcium acetate. Still, it is not surprising that aluminum did not appear in the supernatant liquid resulting from the calcium-acetate treatment. If aluminum had been released from the soil, it would undoubtedly have been precipitated as aluminum hydroxide in the presence of calcium acetate of reaction pH 7.6. If aluminum had entered the alumino-silicic complex of the electrodialyzed soil by replacing its exchangeable hydrogen ions and was not in turn replaceable by calcium, it does not seem that the soil should have adsorbed calcium. But it did adsorb calcium, for 11.1 mgm. equivalents was extracted from the soil by electrodialysis. If the soil had not been previously treated with aluminum acetate, approximately 20 mgm. equivalents of calcium would have been extracted. The effect of the aluminum-acetate treatment was to reduce considerably the adsorptive power of the soil for calcium or to reduce the rapidity of its removal by electrodialysis. This has some relation to the findings of Magistad (5) who has reported that the addition of aluminum to an alkaline soil with which he worked resulted in destroying its capacity to adsorb barium.

TABLE 4

Removal of magnesium and aluminum from soils by calcium acetate and the extraction of calcium from the residual soils by electrodialysis

(Soils electrodialyzed before treatment with magnesium or aluminum acetate)

TREATMENT OF SOIL	MILLIGRAM EQUIVALENTS PER 100 GM. OF AIR-DRY SOIL		
	Mg or Al removed by Ca acetate	Ca in diffusate	Total cations in diffusate in terms of NaOH
Magnesium acetate (pH 7.5)	20.2	19.8	20.2
Aluminum acetate (pH 3.5)	Trace	11.1	11.3

Adsorption of aluminum by soil saturated with hydrogen or calcium

The action of aluminum acetate in reducing the adsorptive power of the electrodialyzed soil for calcium or in retarding its extraction by electrodialysis was an indication that the soil possessed the property of adsorbing aluminum from aluminum acetate when its alumino-silicic complex was saturated with hydrogen and when its hydrogen-ion concentration was approximately 4.0. To test this possibility, an experiment was designed in which 10 gm. of

electrodialyzed soil was treated with 50 cc. of 0.3 *N* aluminum acetate. After the mixture had stood in a closed vessel for 24 hours, aluminum was determined in a part of the clear supernatant liquid. A similar test was conducted with soil which had been electrodialyzed and subsequently saturated with calcium by means of calcium acetate. The results of the experiment are to be seen in table 5.

About 15 mgm. equivalents of aluminum were adsorbed from the solution by the electrodialyzed soil and about 24 mgm. equivalents by the electrodialyzed soil which had been saturated with calcium acetate. Although the calcium-acetate saturated soil contained approximately 20 mgm. equivalents of calcium, only 9.8 mgm. equivalents were extracted by treatment with aluminum acetate. The quantities of calcium, magnesium, and potassium which were extracted from each of the soils will not account for the quantity of aluminum that was removed, and it is not likely that other cations were extracted from the soils in more than minute quantities.

TABLE 5

Removal of aluminum from aluminum acetate by soil saturated with hydrogen or calcium

(Soil electrodialyzed before treatment)

STATE OF SOIL	MILLIGRAM EQUIVALENTS PER 100 GM. OF AIR-DRY SOIL				pH OF SOLUTION BEFORE TREATMENT	pH OF SOLUTION AFTER TREATMENT
	Al removed from solution	Ca removed from soil	Mg removed from soil	K removed from soil		
Hydrogen saturated	14.9	Trace	0.9	1.3	3.78	3.93
Calcium saturated	24.2	9.8	Trace	0.5	3.78	3.85

The process by which aluminum was adsorbed by the soils is not entirely clear. It might be assumed in the case of the acid soil that aluminum replaced hydrogen ions. However, this is not reflected in the hydrogen-ion concentration of the aluminum-acetate solution after it had been in contact with the acid soil. Perhaps it is of some significance that the aluminum adsorbed by the soil treated with calcium acetate, whose reaction was pH 7.4, was essentially equivalent to that adsorbed by the acid soil plus the calcium liberated from the alkaline soil. Thus, it appears that the alkaline soil may have adsorbed as much aluminum as did the acid soil before the calcium was liberated. If the alkaline soil had been treated with a sufficient quantity of aluminum acetate, all of its exchangeable calcium would, no doubt, have been removed.

DISCUSSION

Under the conditions of these experiments and when present individually, potassium, calcium, and magnesium were adsorbed stoichiometrically by electrodialyzed soil and also by soil that had been electrodialyzed and later

made alkaline by treatment with calcium or magnesium acetate. But the manner of their extraction by means of electrodialysis was distinctly different. Although potassium and calcium were removed in equivalent quantities during a period of 8 hours, potassium was removed with greater rapidity than was calcium. The recovery of magnesium was exceedingly slow—less than one half of the quantity known to be adsorbed by the soil was extracted by electrodialysis in 8 hours.

The magnitude of the adsorption of the three cations when added to electrodialyzed soil in molecular equivalent quantities was in the same order as the ease with which they were extracted from the soil by electrodialysis. Thus, more potassium was adsorbed than calcium, and more calcium than magnesium. Although these cations were adsorbed in different quantities, the sum of the quantities so removed was equivalent to that which occurred when each of them was adsorbed in the absence of the other two. Gedroiz (3) found the energy with which barium was replaced from soil by calcium, magnesium, and potassium to decrease in the order named. The present experiment shows potassium to have a greater replacing value than either calcium or magnesium when coexistent in molecular equivalent quantities. No doubt the difference in the order of replacement referred to its attributable to the widely varying conditions under which the two experiments were conducted.

Only an inappreciable quantity of aluminum could be extracted by electrodialysis from electrodialyzed soil that had been treated with aluminum acetate. Nor could larger quantities be extracted in this manner from electrodialyzed soil that had been made alkaline by means of calcium acetate before treatment with aluminum acetate. Although little or no aluminum was extracted from the soil, the soil is known to possess the property of adsorbing aluminum from aluminum acetate when in the two states previously mentioned; that is, when its alumino-silicic complex is saturated with hydrogen or with calcium. When the soil which contained replaceable calcium was treated with aluminum acetate, calcium was liberated. This replacement may have been induced by hydrogen ions rather than by aluminum ions, according to the views of Kelley and Brown (4). But in the case of the acid soil, whose exchangeable cations had been removed by electrodialysis, aluminum may have been adsorbed, not through the precipitation of aluminum as aluminum hydroxide, but in such a manner as to become a part of the colloidal complex of the soil. If this occurred, the effect of the aluminum acetate in reducing the adsorptive capacity of the electrodialyzed soil for calcium might be explained on the assumption that aluminum is held very tenaciously in the colloidal complex of the soil and is removed with difficulty by means of calcium acetate.

Unpublished data obtained in this laboratory have shown that aluminum chloride and aluminum acetate of the same normality react differently with the electrodialyzed soil of the investigation. Aluminum was not adsorbed by the soil when treated with aluminum chloride nor did the treatment interfere more than slightly, if at all, with the subsequent adsorption of calcium by the

soil. Mattson (7) has suggested that soil material does not adsorb aluminum from aluminum chloride because of the acid reaction of the salt or because it renders the soil material electropositive, in which condition it loses its ability to adsorb cations. The failure of the electrodialyzed soil to adsorb aluminum from aluminum chloride was probably due to the acidity of the aluminum chloride induced by the strong chlorine ion. If the treatment had caused the soil to become electropositive it would not have adsorbed calcium, which it accomplished with comparative ease.

Although the process by which aluminum is adsorbed by soils is a matter of conjecture, it does not appear improbable that, under certain conditions, it enters into combination with the colloidal complex of the soil.

It has been suggested by Cooper et al. (2), that a relationship exists between the standard electrode potentials of the elements and the order of their removal from soil by electrodialysis. The relative ease with which the adsorbed cations of the present investigation were recovered by electrodialysis conforms to this suggestion.

SUMMARY

Potassium, calcium, magnesium, and aluminum acetates were added to electrodialyzed soil and also to soil that had been electrodialyzed and subsequently made alkaline by treatment with calcium or magnesium acetate. The rapidity and the comparative extent of their recovery from the soils were ascertained by means of electrodialysis.

The degree to which the cations were extracted from the soil when in either of the states mentioned, was essentially the same. When each cation was adsorbed at the exclusion of the others, potassium and calcium were recovered by electrodialysis in equivalent quantities and to a considerably greater degree than was magnesium. This occurred in spite of the fact that the soil adsorbed as much magnesium as it did potassium or calcium.

Aluminum was adsorbed by the soil when its alumino-silicic complex was saturated with hydrogen or with calcium, but only minute quantities of it could be extracted by electrodialysis. The manner in which it was adsorbed is not clear, but it appears that under certain conditions it might become a part of the alumino-silicic complex of the soil.

Potassium, calcium, and magnesium added to electrodialyzed soil in molecular equivalent quantities were extracted, collectively, to the extent that each of them was extracted when adsorbed individually. When they were adsorbed in the presence of one another more potassium was adsorbed than calcium, and more calcium than magnesium. The ease of their recovery, by electrodialysis, was in the same order as the magnitude of their adsorption.

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THE VALUE OF RAW SEWAGE SLUDGE AS FERTILIZER[1]

J. F. MULLER[2]

Rutgers University

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The disposal of sewage sludge is, in most communities, a problem of vital importance. With the development of the activated sludge system, the use of this type of sludge as fertilizer is coming into favor. There are also a number of disposal plants which sell sludge, as fertilizer, from units in which the sewage solids are decomposed, the decomposition usually amounting to 40–50 per cent of the volatile matter. The sludge, which is sold at a low price, contains about one-half the nitrogen of fresh sewage solids. Little, however, seems to have been done in studying the actual fertilizer value of raw, fresh sludge, dried and ground.

The Atlantic City Sewerage Company, being situated in a community built on white sea sand which is practically devoid of organic matter, felt that there were some interesting economic possibilities in the use of the raw sludge as a soil builder. Hence the study reported in this paper was undertaken.

THE MATERIAL

At the Atlantic City disposal plant the fresh settled solids are pumped daily to sand filter beds. Samples were taken at different times from these beds, allowed to thoroughly air dry, and were then finely ground. The material, after being ground, was very light and fluffy.

These samples were analysed by the official methods of the Association of Official Agricultural Chemists. All analyses were made in duplicate or triplicate. The results are shown in table 1. It is seen that phosphoric acid and potash are present in almost negligible quantities. The carbon-nitrogen ratio is 14.3, which, as is shown in pot series 3, is too wide for optimum plant growth. Chlorides and sulfates are present only in traces.

The total nitrogen content of raw sewage sludge, as shown by analyses of the department of sewage disposal, is usually between 4.5 and 5 per cent. The

[1] The work herein reported was carried on for the Atlantic City Sewerage Co., Atlantic City, N. J., Mr. C. G. Wigley, Engineer; and it is with their kind permission that this paper is published.

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[2] Research Fellow, Department of Soil Chemistry and Bacteriology.

low nitrogen content of the Atlantic City fresh solids might be due to (*a*) a high ash content probably caused by infiltration carrying sand, or (*b*) the type of treatment which allows a good deal of the organic constituents to go through the tanks.

POT EXPERIMENTS

Because the crops growing on an experimental plot at the Atlantic City Sewerage Company plant were destroyed by a heavy northeast storm, this paper deals only with the results obtained in the greenhouse of the department of soil chemistry and bacteriology, New Jersey Agricultural Experiment Station.

TABLE 1

Analysis of dried, fresh sewage sludge

	NUMBER OF ANALYSES	AVERAGE
		per cent
Moisture	4	10.22
Ash	4	37.45
Total carbon	2	28.10
Total nitrogen	5	1.96
Ammonia nitrogen	2	0.12
"Available" nitrogen	2	0.76
Total phosphoric acid	2	0.62
Total potash	1	0.13
Iron and aluminum oxides	1	1.19
pH of water extract	3	5.66

Series 1; Part 1: Beets

Beets were grown in Sassafras sand in glazed earthenware pots. The various fertilizers were mixed with 10 pounds of soil in each case, and in such quantities as to add 225 mgm. of nitrogen to each pot. The plants were thinned to five to each pot two weeks after planting, and harvested two months after planting. The results are given in table 2, the green weights being referred to a base of the checks equal to 100. The results are the averages of duplicate treatments. The fertilizer mixtures were made up as shown in table 3.

Series 1; Part 2: Barley

After the beets were harvested, the pots were allowed to stand fallow for three weeks. Barley was then planted and was harvested two months later. The results are given in table 2 under "Barley," the figures being relative green weights of tops. It should be pointed out that although the checks are rated at 100 in the cases of the beets, barley, and combined crops, and the rest of the weights are referred to those bases, the actual weights in all three cases were, of course, widely different.

The principal facts brought out in table 2 are: first, the mineral fertilizer increased the beet growth greatly over that of the check, but was largely used up by this crop, and the residual crop, barley, made comparatively little better growth than the check; second, the sludge had practically no beneficial action when used alone on either beets or barley, and in the case of the barley where no lime was used, actually decreased the yield below that of the check; third, it appears that phosphoric acid is a limiting factor, in the case of beets at least, since mixture IV containing no phosphate gave very poor growth.

TABLE 2

Beets and barley

POT NUMBER	TREATMENT	RELATIVE GROWTH		
		Beets	Barley	Combined crops
	gm.			
1	Nothing (check)	100	100	100
2	4.5 5-8-5 mineral mixture	4,380	127	698
3	4.5 5-8-5 mineral mixture	.....	...	...
	4.3 lime	5,340	108	810
4	15.0 dried sludge	110	63	70
5	15.0 dried sludge	.....	...	...
	4.3 lime	200	111	123
6	7.5 sludge mixture I	850	168	259
7	6.5 sludge mixture II	1,030	123	245
8	5.0 sludge mixture III	540	141	195
9	5.5 sludge mixture IV	30	64	59

TABLE 3

Fertilizer mixtures

(Parts by weight)

MIXTURE	ANALYSIS	NITRATE OF SODA	SUPER-PHOSPHATE	MURIATE OF POTASH	DRIED SLUDGE
Mineral..........................	5-8-5	3.0	5	1.0	..
Sludge I..........................	3-5-2	3.0	6	1.0	10
Sludge II..........................	3-8-4	4.5	10	1.5	4
Sludge III..........................	4-7-4	(5 nitro-phoska; 5 gypsum)			10
Sludge IV..........................	4-0-3	3.8	..	1.2	10

Series 2: Beets

In series 2 the same kind of soil was used as before, and beets were again the crop grown. Ten pounds of soil to each pot was used. Table 4 gives the treatments and the results, relative green weights referred to the check as 100.

The treatment in pot 2 was at the rate of 10 tons of dried sludge to the acre. The results of this series indicate that a sufficiently heavy application of sludge is about as efficient as sludge supplemented by phosphate or potash. Considerable benefit was derived from the application of nitrogen.

Series 3: Beets

Series 3 was planned to determine the effect of narrowing the carbon-nitrogen ratio of the dried sludge by the addition of available nitrogen in the form of nitrate of soda. The same kind of soil was used as in series 1 and 2, and beets were again planted. Superphosphate and muriate of potash were added to all pots alike, including the check. Four mixtures were prepared having carbon-nitrogen ratios varying from 13.3 to 3.3. White quartz sand was added to give a uniform proportion of sludge to minerals in all of the mixtures. The treatments and results, relative green weights on the basis of the check as equal to 100, are shown in table 5.

TABLE 4
Beets

POT NUMBER	TREATMENT	RELATIVE GROWTH
	gm.	
1	Nothing (check)	100
2	45 dried sludge	250
3	15 dried sludge, 2 nitrate of soda	340
4	15 dried sludge, 5 superphosphate	220
5	15 dried sludge, 2 muriate of potash	230

TABLE 5
Beets

POT NUMBER	TREATMENT	N:C	N	RELATIVE GROWTH
			mgm.	
1	15 gm. 15 parts sludge	1:13.3	208.5	100
	5 parts sand	......	.....	...
2	15 gm. 15 parts sludge	1: 8.6	321.0	210
	1 part nitrate	......	.....	...
	4 parts sand	......	.....	...
3	15 gm. 15 parts sludge	1: 5.0	546.0	250
	3 parts nitrate	......	.....	...
	2 parts sand	......	.....	...
4	15 gm. 15 parts sludge	1: 3.3	846.0	230
	5 parts nitrate	......	.....	...

The results indicate that a narrowing of the carbon-nitrogen ratio to about 8 is desirable, but uneconomical below this. This would explain the stimulating effect of the added nitrogen in series 2, since there the ratio was reduced to 1:8.

Series 4; Part 1: Grass

A quantity of sea sand was obtained from Atlantic City for series 4, and a commercial lawn grass seed was planted in it. After an initial vigorous growth

had been made, the grass was clipped periodically over a period of several weeks, and the clippings were weighed. Table 6 gives the treatments, and the results in actual green weights of the grass clippings.

Series 4; Part 2: Grass

This series paralleled part 1, the same kind of soil and seed being used. A sample of sludge was obtained which had been subjected to the action of fungi in a closed box for several days before being dried and ground. The treatments and results are shown in part 2 of table 6.

It is apparent from the results of this series that dried, ground, fresh sewage sludge is of considerable value as a turf dressing, giving a better growth than an application of mineral fertilizer. The use of minerals as a supplement to the sludge, in the case of grass, would seem to be uneconomical. The fungus

TABLE 6

Grass

POT NUMBER	TREATMENT	GREEN WEIGHTS
	gm.	*gm.*
	Part 1	
1	20 sludge	28.00
2	20 sludge, 1.1 superphosphate	23.15
3	20 sludge, 2.0 minerals (5–8–5)	28.25
4	2 minerals (5–8–5)	19.95
5	Nothing (check)	12.75
	Part 2	
6	Nothing (check)	12.25
7	20 sludge	19.10
8	20 sludge (fungus treated)	20.40

treatment of the sludge is shown by the experiment to be of no benefit in bringing the plant-food contained in the sludge into a more available form.

Series 5: Corn

Sweet corn was grown in Sassafras sand, 10 pounds of soil to the pot, and the moisture was maintained by periodic weighings and replenishments. The germination in all cases was excellent. Two weeks after planting, the plants were thinned to six a pot; then three and one-half weeks after planting three plants were removed from each pot and weighed; five and one-half weeks after planting two more were removed; and the last remaining plant was harvested nine weeks after planting. The treatments and the green weights, average for each plant of those removed from each pot, are given in table 7.

The heights were measured weekly, by drawing up the longest leaves, the results being shown in table 8. The apparent decrease in height of some of the plants during the last weeks of the experiment was due to the shriveling of the ends of some of the leaves, and not to a dying back of the crown of the plant. At this time upward growth had practically ceased, and the plants were in most cases in tassel, and some, for example those in pots 13, 14, and 16, were forming ears.

TABLE 7

Corn

POT NUMBER	TREATMENT	GREEN WEIGHTS		
		3½ weeks	5½ weeks	9 weeks
	gm.	*gm.*	*gm.*	*gm.*
1	Nothing (check)	3.81	8.38	12.85
2	2 lime	3.33	6.25	13.75
3	2¼ minerals (5-8-5)	5.40	13.15	51.55
4	2¼ minerals (5-8-5)	....	.....	.....
	2 lime	5.39	10.40	29.25
5	45 sludge	3.96	7.35	15.60
6	45 sludge, 2 lime	3.32	7.18	12.78
7	90 sludge	3.13	7.98	17.68
8	90 sludge, 2 lime	4.02	6.28	13.15
9	45 sludge	....	.....	.....
	½ sulfate of ammonia	4.88	8.05	20.10
10	45 sludge	....	.....	.....
	2½ sulfate of ammonia	3.82	7.08	14.40
11	45 sludge	....	.....	.....
	2½ sulfate of ammonia	....	.....	.....
	2 lime	4.10	9.03	18.25
12	45 sludge	....	.....	.....
	1½ superphosphate	3.65	9.80	51.80
13	45 sludge	....	.....	.....
	5¼ superphosphate	4.05	10.80	68.80
14	45 sludge	....	.....	.....
	2½ sulfate of ammonia	....	.....	.....
	5¼ superphosphate	3.25	16.08	89.26
15	45 sludge	....	.....	.....
	½ muriate of potash	3.96	10.15	16.15
16	45 sludge	....	.....	.....
	2¼ minerals (5-8-5)	5.05	14.33	81.80

The results of this experiment are, in general, similar to those already found in the preceding series. Medium or light applications of sludge alone are of practically no benefit, whereas heavy applications increase plant growth to some extent, as is shown by treatments 7 and 8.

The conclusion drawn from series 1, that a phosphate supplement is desirable, is further confirmed by treatments 12 and 13 of this series, and by the large

increase in growth of 14, which received sludge supplemented by superphosphate and sulfate of ammonia, over 10 and 11, which received the same

TABLE 8

Height of corn in inches

POT NUMBER	WEEKS								
	1	2	3	4	5	6	7	8	9
1	1.97	8.48	13.90	18.70	20.50	21.88	22.13	22.00	22.00
2	2.04	8.21	13.35	18.40	19.90	22.00	22.38	22.25	22.50
3	2.31	8.86	14.55	19.75	26.70	29.75	31.50	32.25	33.50
4	2.23	9.25	15.30	21.00	24.80	29.00	31.38	32.13	32.50
5	2.44	9.02	14.20	17.10	19.40	21.75	22.60	22.73	22.50
6	2.13	8.90	14.25	18.80	21.40	21.75	22.25	22.13	21.50
7	2.38	9.30	14.40	18.95	21.70	23.50	24.50	25.25	25.25
8	2.16	8.79	13.67	17.05	20.00	23.38	24.50	24.63	24.50
9	2.63	9.98	15.10	19.60	21.60	22.28	24.13	24.00	23.75
10	2.52	8.77	14.10	19.20	21.80	24.25	25.63	26.25	26.00
11	2.68	9.48	15.15	19.75	22.50	24.00	25.75	26.00	25.50
12	1.64	8.48	13.71	18.85	22.80	26.88	30.50	32.35	33.50
13	2.34	9.38	14.15	18.80	23.00	31.50	32.50	33.75	36.50
14	2.15	8.71	13.85	21.30	26.40	28.50	39.75	42.13	46.25
15	2.23	9.63	15.20	20.80	24.70	26.75	28.00	27.50	27.00
16	2.75	9.86	16.45	21.90	26.70	32.25	34.25	37.00	38.50

TABLE 9

Loss of moisture in pounds

POT NUMBER	DATES										TOTAL
	12/31	1/10	1/18	1/22	1/26	2/2	2/8	2/14	2/20	2/27	
1	0.25	0.83	0.55	0.45	0.35	0.78	0.60	0.38	0.60	0.80	5.59
2	0.25	0.73	0.53	0.38	0.23	0.63	0.55	0.33	0.50	0.73	4.86
3	0.25	0.70	0.63	0.48	0.40	1.03	1.00	0.63	0.98	1.23	7.33
4	0.28	0.78	0.60	0.48	0.38	0.95	0.88	0.53	0.85	1.10	6.83
5	0.23	0.63	0.50	0.38	0.33	0.70	0.60	0.35	0.45	0.68	4.85
6	0.20	0.60	0.45	0.35	0.28	0.63	0.55	0.23	0.40	0.58	4.27
7	0.18	0.38	0.43	0.28	0.33	0.70	0.60	0.33	0.45	0.65	4.33
8	0.18	0.50	0.43	0.33	0.30	0.68	0.58	0.26	0.38	0.60	4.24
9	0.28	0.70	0.65	0.50	0.40	0.90	0.73	0.35	0.55	0.68	5.74
10	0.23	0.63	0.53	0.43	0.30	0.70	0.65	0.35	0.53	0.68	5.03
11	0.10	0.48	0.50	0.43	0.35	0.83	0.65	0.36	0.43	0.63	4.76
12	0.20	0.65	0.63	0.40	0.35	0.95	0.93	0.58	1.03	1.30	7.02
13	0.23	0.68	0.63	0.40	0.35	1.03	1.03	0.65	1.13	1.38	7.56
14	0.20	0.58	0.70	0.50	0.50	1.23	1.18	0.83	1.28	1.38	8.38
15	0.20	0.68	0.55	0.53	0.35	0.80	0.65	0.30	0.45	0.63	5.14
16	0.23	0.73	0.70	0.55	0.40	1.18	1.05	0.65	1.20	1.40	8.09

amounts of sludge and ammonia but no phosphate. The mixture in treatment 14 is equivalent on a ton basis to 1700 pounds of dried fresh sludge, 100 pounds

of sulfate of ammonia, and 200 pounds of 20 per cent superphosphate. It is applied at the rate of approximately 12 tons an acre.

The large differences between the heights and weights of the plants in pot 3 and those in pot 16, receiving minerals, and sludge and minerals, respectively, are especially noteworthy, inasmuch as the same mineral treatment was used in each case.

It should be borne in mind that the larger corn plants transpire very considerable quantities of water, and in interpreting table 9, it is necessary to refer to tables 7 and 8 and for comparison select treatments giving similar growth. Thus by comparing pot 1, receiving no sludge, with 5 receiving 45 gm. of sludge and 7 receiving 90 gm. of sludge, all of which show a comparable growth, we find that the loss of moisture is in the inverse order; namely, 1 lost 5.59 pounds, 5 lost 4.85 pounds, and 7 lost but 4.33 pounds. By calculating this to an acre basis, 1 lost 487,000 pounds of water an acre, 5 lost 422,532 pounds, and 7 lost 377,229 pounds. The difference between that lost by 7 and that lost by 1 is the equivalent of 0.5 inches of rainfall in the period of the experiment, which was nine weeks. This is of very considerable importance in a locality where the soil is so predominantly coarse sand.

This series has shown little benefit from the use of lime with the sludge. In fact where parallel treatments are used with and without lime, the lime has slightly depressed growth in all cases as measured by the heights of the plants, and in all but one case where measured by green weights.

DISCUSSION

From the results of the experiments herein reported, it would seem that dried fresh sewage sludge could be profitably employed as a fertilizer material, and especially as a soil builder. Although light applications did not appear to be of any benefit to plant growth, series 2 and series 5, beets and sweet corn, both heavy feeders, showed that when used at the rate of approximately 10 tons or more an acre, the dried fresh sludge will considerably increase plant growth without the use of any mineral fertilizer supplements.

As is to be expected from the nature of the material and its method of treatment, only the insoluble and hence slowly available nitrogen is left in the dried sludge. Because of the high content of carbonaceous material and this lack of available nitrogen, the decomposition of the sludge in the soil will deplete the supply of the available soil nitrogen at the expense of higher plants. The coincidental application of some nitrogen carrier such as nitrate of soda or sulfate of ammonia with the dried fresh sludge will therefore not only give the crop a quick start but will also increase the rapidity of the decomposition of the sludge.

The desirability of the use of a phosphate supplement in conjunction with dried fresh sewage sludge is indicated both by the analysis and by the experiments. A phosphate fertilizer is required under almost all soil conditions and for most crops, and since the sludge used was almost devoid of this plant-

food, it is not surprising that the supplementing of the sludge with superphosphate should give a large growth response.

Potash on the other hand, seldom gives as marked an increase in plant growth as nitrogen and phosphorus, and this has been the case in the experiments reported herein. While many soils contain a considerable quantity of potash, more-or-less available, it is very likely that potash will increase growth sufficiently on the sandy soil of the Atlantic City region to warrant its use in conjunction with the dried fresh sewage sludge.

These experiments would indicate, therefore, that to obtain the best and most efficient results from the use of dried fresh sludge, it should be supplemented with available nitrogen, phosphorus, and potash carriers. A mixture suggested by the results of the experiments would be 1650 pounds of dried, ground fresh sewage sludge, 100 pounds of sulfate of ammonia, 200 pounds of 20 per cent superphosphate, and 50 pounds of muriate of potash, to make a ton. The carbon-nitrogen ratio in such a mixture would be approximately 6, which is well within that found to be desirable by experiment (series 3).

As was pointed out in the discussion of series 4, grass, no mineral supplement appears to be necessary to give a good turf growth. It is well known that grass requires large amounts of moisture for satisfactory continuous growth. It is highly probable that the chief benefit from the use of dried fresh sludge on grass, especially where the soil is so extremely sandy, is the increasing of the water-holding capacity and the prevention of the rapid drying of the surface soil where the grass roots are concentrated. This has been clearly brought out in table 9.

Although the experiments have shown variable results from the use of lime with the sludge, it is quite certain that if this material were used on the same soil year after year, a considerable acidity would be developed, and lime would become necessary. This would be especially true if sulfate of ammonia were used in conjunction with the sludge.

It is of course freely admitted that in order to obtain thoroughly conclusive results and to determine what the action of dried fresh sewage sludge will be under varying soil and climatic conditions and its effect on large numbers of widely different types of plants, a series of experiments run continuously for several years would be necessary. It is felt however, that the experiments described in this paper have sufficiently proved that dried fresh sewage sludge has considerable fertilizer value when used under proper conditions. Nothing has been said regarding the mechanics or the economics of the grinding and drying processes, as that is an engineering problem.

SUMMARY

Several samples of dried fresh sewage sludge were analyzed, and pot experiments were carried out to determine the fertilizer value of this kind of material.

The analyses showed considerable potential plant-food to be present.

The narrowing of the carbon-nitrogen ratio to below 8 by the addition of available nitrogen largely increased the fertilizer value of the sludge.

A phosphate supplement appears to be necessary for good plant growth, and a potash supplement, in small quantities, would seem desirable.

The dried sludge alone, without mineral supplements of any kind, when applied to turf grown on sand gave a good stand of grass and prevented its dying off.

The application of dried fresh sludge to a sandy soil increased its water-holding capacity very materially, which is a most desirable result on soils of this type.

Although the experiments reported did not indicate a marked necessity of using lime with the sludge, it is almost certain that lime would be required after several years continuous application of sludge.

STUDIES OF NITROGEN FIXATION BY THE ROOT NODULE BACTERIA OF THE LEGUMINOSAE[1]

E. W. HOPKINS

Wisconsin Agricultural Experiment Station

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It is now more than forty years since Beijerinck published his memorable paper concerning the bacteria in the rot nodules of the *Leguminosae*. During these four decades there have appeared in the scientific literature some 46 papers which deal to a greater or less extent with the problem of nitrogen assimilation by means of these nodule-forming bacteria. Although many careful studies have been made, it must be admitted that as yet no satisfactory evidence has been forthcoming to explain the part played by the bacteria in this assimilation of nitrogen by leguminous plants. The negative results, or almost negative, of so many investigators have been the stumbling block in the path of many bacteriologists. It is unfortunate that as yet no one has devised a satisfactory apparatus or medium which would greatly favor the assimilation of nitrogen by the bacteria in the absence of the host plant.[2] It may not be out of place to include a statement from Dible: "It is a natural characteristic of research workers in all countries that they publish their positive findings but often relegate their negative ones to oblivion." Such is not the case with this report. The results are mainly negative. Certain investigators report well-defined gains whereas others are unable to find any increase in combined nitrogen. In table 1 their findings are summarized. The column designated "Number of analyses," is an approximate estimate of the total number. The figures do not include any preliminary analyses. In the column "Nitrogen fixed," an effort was made to reduce all results to a common basis, milligrams of nitrogen in 100 cc., and in the last column on the right to give the author's conclusions regarding his results.

Many of these reports fail to give complete information regarding the medium, the cultures used, and the results of the analyses. These papers need not be discussed, inasmuch as the essential data are given in the table. In some cases there is doubt about the purity of the cultures. For example, there is little doubt that Mazé's cultures (35, 36) were impure because the author speaks of the "cheesy" odor of his cultures and because sufficient pre-

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] Dible, J. H. 1929 Recent Advances in Bacteriology. Philadelphia.

TABLE 1

A summary of reports upon the fixation of atmospheric nitrogen by root nodule bacteria in culture

	MEDIUM USED	NUMBER OF ANALYSES (ESTIMATED)	NITROGEN FIXED IN 100 CC. OF CULTURE	REPORT
			mgm.	
Beijerinck (4)	Nitrogen free salts, asparagin agar	....	.....	No
Prazmowski (41)	Nitrogen-free medium	....	.....	Yes
Beijerinck (5)*	Bean seedling extract + sucrose + KH_2PO_4	6	0.9–1.8	Probable
Frank (13)	Nitrogen-free medium	....	.....	Uncertain
Immendorff (30)	Various kinds	....	None	No
Berthelot (7)	"Cohn's medium" + humic acid	....	5.3‡‡	Yes
Gonnermann (19)	Potato pulp	....	None	No
Heinrich (25)	Potato pulp	19	None	No
Stutzer, Burri, and Maul (51)*	Potassium phosphate, $MgSO_4$, NaCl, $CaCl_2$, and glucose solution	8	6.0	No
Mazé (35)*	Bean seed extract + NaCl + $NaHCO_3$ + sucrose, with and without agar	6 (?)	23.4–27.1	Yes
Zinsser (53)	Potassium phosphate, $MgSO_4$, "sugar" solution	....	None	No
Mazé (36)*	Bean seed extract + sucrose	3	24.2–30.0	Yes
Stoklasa (50)	.....	....	None	No
Grieg-Smith (20)	Lupine leaf extract + KH_2PO_4 + $CaCl_2$ + agar	....	.....	No
Grieg-Smith (21)	Various media, including Mazé's	....	None	No
Neumann (38)*	Plant extract and peat extract	9	4.4–49.9	Yes
Hiltner and Störmer, (28)	Glucose, KH_2PO_4, and peptone, asparagin or KNO_3 solutions	....	None	No
Chester (00)§§	K_2HPO_4, NaCl, $FeSO_4$, $CaCO_3$, glucose agar	8	0.6–2.5	Yes
Lewis and Nicholson (33)†	Sucrose salt solutions and sucrose bouillon	30	0–16.2	Yes
Löhnis (34)‡	Soil extract + glucose + K_2HPO_4	....	2.8–3.6	Yes
Moore (37)*	Maltose, $MgSO_4$, potassium phosphate solution	90	0.2–2.2	Yes
Golding (17)	Bean and pea plant juices + sugars, K_2HPO_4, NaCl, $FeSO_4$, $MnSO_4$, $MgSO_4$ and succinic acid	10	2.1–3.5 (with pure cultures.)	
Grieg-Smith (22)§	Glucose, sodium phosphate solution + agar	56	1.0–4.0	Yes

TABLE 1—*Continued*

	MEDIUM USED	NUMBER OF ANALYSES (ESTIMATED)	NITROGEN FIXED IN 100 CC. OF CULTURE	REPORT
			mgm.	
Budinov (11)*	Dilute bean extract + sucrose	2	4.1	Yes
Bottomley (8)	KH_2PO_4, NaCl, $CaCO_3$, $FeSO_4$, mannite solution	6	0.4	Yes
de'Rossi (43)**	Bean seed and bean and vetch leaf extracts + sugars, NaCl, solutions or + gelatin or agar	33	−27.7–3.1 per 100 gm.	No
Fred (14)*	K_2HPO_4, $MgSO_4$, NaCl, $Fe_2(SO_4)_3$, $CaCl_2$, $MnSO_4$, glucose solution	....	0.18–1.68	Yes
Bottomley (9)	Same as Bottomley 1909	....	1.8	Yes
Golding (18)	.....	....	.....	Yes
Fred (15)*	Same salts as Fred 1909–10 + maltose and sucrose + agar	101	0.15–1.66	Yes
Bottomley (10)	$MgSO_4$, potassium phosphate, maltose solution	....	2.0	Yes
Grieg-Smith (23)	?	....	3–5.6	Yes
Spratt (46, 47)*	$MgSO_4$, potassium phosphate, sucrose solution	8	2.5–3.5	Yes
Herke (26)	Soil extract + K_2HPO_4 + mannite	37	0.14–1.2	Yes
Olaru (40)	Mazé's bean extract + Mn.	22	1.5–32.1	Yes
Rocasolano (42)*	Mannitol solution + Mn.	8	2.1–9.6	Yes
Beijerinck (6)	K_2HPO_4, lime, glucose solution + garden soil; other media	....	.....	No
Hills (27)†	$MgSO_4$, KH_2PO_4, NaCl, $CaSO_4$, $CaCO_3$, mannite agar	46	0.15–3.5	Yes
Joshi (32)	Soil extract + K_2HPO_4 + mannite	....	0.8–2.0	Yes
Singh (44)*	Soil and sucrose, K_2HPO_4 solution	42	0.25–10.75	Yes
Hutchinson (28*a*)	.....	....	2.5	Yes
Voicu (52)*	Bean seed extract + sucrose + Boron	45	2.4–3.5	Yes
Hutchinson (29)	.....	....	.....	Yes
Barthel (2)††	KH_2PO_4, $MgSO_4$, NaCl, $CaSO_4$, KNO_3, $FeCl_3$, mannite solution	8	−0.21–0.39	No

TABLE 1—*Concluded*

	MEDIUM USED	NUMBER OF ANALYSES (ESTIMATED)	NITROGEN FIXED IN 100 CC. OF CULTURE	REPORT
			mgm.	
Fred, Whiting, and Hastings (16)†	Soil extract + sucrose	4	0.1	Yes
Allison (1)*	Clover plant extract + glucose	....	None	No
Bazarewski (3)	Synthetic medium	....	1.3–3.0	Yes
Stiehr (48)	Lupine extract-glucose agar	5	0.6–2.8 per 100 gm.	Yes
Halversen (24)§	Moore's dextrose-Ashby's solution	....	0.2–9.86	Yes
Skinner (45)§	Ashby's agar	4 (?)	None	No

When no note is made, the method of analysis was not designated.
* Kjeldahl.
† Gunning modified to include nitrates.
‡ Kjeldahl-Wilfarth.
§ Kjeldahl-Gunning.
** Kjeldahl-Jodlbauer.
†† Bristol-Page.
‡‡ 5.3 mgm. gained in total culture. Volume not given.
§§ Chester, F. D. 1904 Soil bacteria and nitrogen assimilation. Del. Agr. Exp. Sta. Bul. 66.

cautions were not taken to sterilize the air with which the cultures were aerated. In spite of this fact, Mazé's experiments are often quoted as proof that the root nodule bacteria are capable of fixing nitrogen outside of the host plant. What appear to be the best papers are briefly summarized below.

Beijerinck was the first to test the nitrogen-fixing ability of the root nodule bacteria. In 1888 (4), he found that bacteria from the nodules of *Vicia faba* fixed no nitrogen in a solution of salts to which asparagin had been added. Organisms from the nodules of *Cytisus laburnum* fixed no nitrogen on a solid medium. Again in 1891 (5), the nodule bacteria were grown in bean seedling extract to which sucrose (1.5 to 2 per cent) and varying amounts of KH_2PO_4 were added. Analyses made by the Kjeldahl method showed a slight gain in nitrogen (0.9 to 1.8 mgm. N in 100 cc.). The author concluded that nitrogen fixation was probable, but was not satisfied that his experiments had established fixation. A further report in 1918 (6) stated that only a very slight fixation had occurred in a synthetic medium. Plant extracts containing 2 per cent sucrose gave a slight "but not convincing" fixation of nitrogen. The author at that time concluded that nitrogen is not fixed by the root nodule bacteria. Beijerinck was very careful throughout this work to examine his cultures for purity, so that on this point one feels secure.

Findings similar to those of Beijerinck are reported by a number of investigators; namely, Immendorff (30), Gonnermann (19), Heinrich (25), Zinsser (53), Stoklasa (50), and Grieg-Smith (20, 21).

The following quotation from Nobbe and Hiltner (39) very clearly expresses the opinion of certain investigators on the question of nitrogen fixation by nodule bacteria:

"Trotz vielfacher Versuche ist es bisher nicht mit Sicherheit gelungen, durch Kultur des *Bacterium radicicola* in den verschiedensten Medien eine in Betracht kommende Zunahme des Stickstoffgehaltes zu erzielen."

Stutzer, Burri, and Maul (51) grew the nodule bacteria of alfalfa in a glucose mineral-salt solution and placed long-fibred asbestos in each flask. Although a gain in nitrogen of 6 mgm. in 100 cc. was found, no fixation was reported.

Soil extract with 1 per cent of glucose and 0.05 per cent K_2HPO_4 was used by Löhnis (34) for the growth of bacteria of clover and vetch nodules. The soil extract contained no nitrates, but in order to determine the nitrates which he states were absorbed from the air, a nitrogen method was used to include the nitrate nitrogen, the Kjeldahl-Wilfarth procedure. Gains in nitrogen of 2.8 to 3.6 mgm. in 100 cc. were found.

Moore (37) reports gains of nitrogen of 0.2 to 2.2 mgm. in 100 cc. of medium with red clover, soybean, white lupine, hairy vetch, berseem, and garden pea root nodule bacteria. A nitrogen-free solution of magnesium sulfate, potassium phosphate, and maltose was used, and the cultures were aerated.

Gains of like amounts of nitrogen are reported by Grieg-Smith (22), Bottomley (9, 10), Spratt (46, 47), Joshi (32), Hutchinson (28*a*), Bazarewski (3), and Stiehr (48).

Golding's work (17) is usually quoted with that of Mazé as proof that the root nodule bacteria fix atmospheric nitrogen. Golding attempted to remove the soluble products, by growing his cultures in an inverted and covered bell-jar, and drawing off the culture medium through a porous filter-candle at the bottom. Apparently the medium was not added continuously, and thus the conditions of the experiment were quite different from those existing in the plant.

In one experiment unheated, macerated young bean plants were placed in the inverted bell-jar with distilled water, and the apparatus attached to the suction pump. A gain of 11.4 mgm. of nitrogen in 100 cc. took place in 15 days. In later experiments the medium was sterilized and inoculated with pure cultures of root nodule bacteria (strain not mentioned). The gain in nitrogen amounted to about 2.1 and 3.5 mgm. of nitrogen in 100 cc.

De'Rossi (43) attempted to duplicate the results of Mazé, but the gains of nitrogen in 100 gm. of agar medium were: + 1.0 mgm., −27.7 mgm., +2.0 mgm. Other results were +3.1 mgm., −7.7 mgm. An experiment was also set up similar to that of Golding but showed the following results: +4.2 mgm., −3.4 mgm. N in 100 cc. This worker concluded that the nodule bacteria fix only insignificantly small amounts of nitrogen.

Olaru (40) used Mazé's medium for the growth of the root nodule bacteria, but added varying amounts of manganese salts. When no manganese was present in the medium, a fixation of 2.0 mgm. of nitrogen in 100 cc. took place,

but in one case when 0.5 mgm. of manganese was present, the gain in nitrogen was 32.1 mgm. Rocasolano (42) also reports that manganese has a stimulating effect on nitrogen fixation.

The effect of nitrates on the fixation of nitrogen by the nodule bacteria was studied by Hills (27). Varying amounts of calcium, potassium, and sodium nitrates were added to the agar. The nitrogen was determined by the modified Gunning (salicylic acid) method to include the nitrates. A fixation of 0.15 to 3.5 mgm. of nitrogen in 100 cc. is reported. However, the salicylic acid method has been found by Davisson and Parsons (12) to be unreliable for the determination of nitrate-nitrogen in the presence of water.

Barthel (2) obtained no fixation by pea organisms in a nitrogen-free mineral salts solution with and without caffeine added. Red clover plant extract, according to Allison (1), gave no gain in nitrogen when inoculated with red clover nodule bacteria.

In dextrose- Ashby's solution cultures of *Rh. leguminosarum*, Halversen (24) reported gains of 0.2 to 9.86 mgm. in 100 cc.

From the lack of agreement shown in the work cited, it is evident that the question of the fixation of nitrogen by the root nodule bacteria outside of the host plant is by no means settled. Many of the discrepancies can be explained on the basis of impure cultures, or method of analysis, but the variable factors such as the stimulating effect of certain chemical elements, the influence of the age of the inoculum, the differences in the amounts of nitrogen supplied the bacteria, the possible stimulating effect of plant extracts, do not admit the reduction of all these results to a common basis.

It was, then, in the hope that further experiments in which better methods were used would bring order out of the chaos, that this problem was undertaken.

EXPERIMENTAL

Preparation of media

Usually, a soil high in organic matter was heated for half an hour at 120°C. with an equal weight of water. Calcium carbonate (10 gm. per kilo of soil) was stirred into the hot soil sludge, and the liquid filtered from the soil. To the clear, pale yellow extract, 1 per cent of sugar (glucose or sucrose) and 0.05 per cent K_2HPO_4 were added. One hundred cubic centimeters of this medium was put into 750 cc. Erlenmeyer flasks, the flasks plugged with cotton and sterilized.

Culture methods

The cultures used for the inoculation were grown on slants of yeast water mannitol agar, and were 3 to 6 days old when used. The controls were inoculated in the same way as the culture flasks and were then heated for 10 to 25 minutes at 120°C., in an autoclave. The purity of all cultures used was tested before analysis.

Cultures were incubated at room temperature (20 to 25°C.) unless otherwise stated. Both young and old cultures were analyzed to determine the influence of age on the fixation of nitrogen.

Methods of analysis

The nitrates, which were invariably found present in the soil extracts, made necessary the use of a method of analysis which would include this form of nitrogen as well as the organic and ammonia nitrogen. If the nitrate nitrogen were lost in analysis, the nitrogen present in this form in the controls would be driven off, whereas in the inoculated cultures the nitrates might have been used by the bacteria and converted into protein. An apparent fixation would be the result of such an occurrence. The salicylic acid method (Gunning method modified to include nitrates) had been shown by Davisson and Parsons (12) and by Jacob and Geldard (31) to be unreliable for the determination of nitrates in the presence of water. The Davisson-Parsons method was used, as it appeared to be the most reliable and convenient method of nitrogen determination to include nitrates.

Titrations were made with 1/28 N NaOH and H_2SO_4.

Experiment 1

In 1927 a number of experiments dealing with the problem of nitrogen fixation by the nodule bacteria in pure culture were made by Miss M. P. Löhnis. As a culture medium, among others, soil extract with mannitol or glucose added was used, and the nitrogen analyses were made by the Gunning method modified to include nitrates. A large number of positive results were obtained, and those in which soil extract was used are given in table 2.[3] The cultures studied included Dalea 901, Bean 402, Red Clover 205, Sweet Clover 115, Soybean 501, and Pea 317 and 302. A fixation of -0.27 to $+2.13$ mgm. of nitrogen in 100 cc. seems to have taken place. All cultures except Soybean 501 showed a small gain of nitrogen. One set of cultures of Red Clover 205 and Pea 317 were aerated with air passed through cotton, acid, and alkali towers. The apparent fixation as shown in the figures of this table is, no doubt, due to the faulty method of analysis.

As the soil extract contained nitrates, it was deemed necessary to check these results with the more reliable method of Davisson-Parsons.

Experiment 2

The total nitrogen of the soil extract prepared by the method described in the foregoing was determined by the Davisson-Parsons method. Although copper is present in the Devarda's alloy used in this method for the reduction of the nitrates, it was feared that insufficient amounts were present to catalyze

[3] A more detailed description of these experiments will be published elsewhere.

completely the oxidation of the organic matter. Three 100-cc. portions of soil extract were analyzed by the Davisson-Parsons method, and to three others 0.7 gm. HgO was added, the procedure being otherwise identical with that recommended by Davisson and Parsons. Triplicate analyses made in

TABLE 2

The gain and loss of nitrogen by soil extract cultures of root nodule bacteria

ORGANISM	NUMBER OF CULTURES SHOWING		GAIN OR LOSS IN NITROGEN	
	Gain in N	Loss in N	Maximum	Average
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
Pea 317	16	2	2.26	1.06
Pea 302	2	0	1.13	0.99
Red Clover 205	18	0	3.42	1.75
Bean 402	12	2	3.19	1.38
Sweet Clover 115	7	3	2.40	0.74
Soybean 501	3	6	2.00	−0.27
Dalea 901	17	0	3.33	2.13

TABLE 3

Analyses of cultures of Dalea 901 in soil extract

(Second experiment. Age 11 days)

CONTROL NUMBER	TOTAL NITROGEN	CULTURE NUMBER	TOTAL NITROGEN
	mgm. per 100 cc.		*mgm. per 100 cc.*
1	2.1	37	2.2
2	2.1	38	2.1
3	2.3	39	2.4
4	2.3	40	2.1
5	2.4	42	1.6
6	2.3	43	1.9
7	2.1	44	1.7
8	2.0	45	2.0
		46	2.1
		47	2.1
		48	2.1
		49	2.0
		50	2.0
		51	2.0
		52	1.9
Average	2.2		2.0

these two ways gave identical results, indicating that HgO was not necessary. The total nitrogen content of this soil extract averaged 2.1 mgm. per 100 cc.

The nutrient materials, K_2HPO_4 and glucose, were added to the soil extract and 100-cc. portions of the medium sterilized in 750-cc. Erlenmeyer flasks These flasks were inoculated with three drops of a suspension of 3-day-old

root nodule bacteria of known pure cultures. The organisms used in this experiment were: Dalea 901, Alfalfa 100, and Red Clover 205.

The flasks were incubated at room temperature. Eleven days after being inoculated, all Dalea 901 cultures were tested for purity, and they, with eight controls, were analyzed for total nitrogen. The analytical results are given in table 3. Apparently, a slight loss of nitrogen had taken place.

Sugar analyses by the micro method of Stiles, Peterson, and Fred (49) of 35-day-old cultures of Red Clover 205 and Alfalfa 100 indicated that very little fermentation (5 to 9 per cent) had taken place. This was no doubt due to the unfavorable reaction of the medium, which was pH 4.6 to 5.2. These cultures failed to gain in nitrogen. In view of the small amount of sugar fermented, the results are not included in the tables.

Experiment 3

In order to obtain a soil extract higher in nitrogen content than that used in the last experiment, the soil-water mixture was heated for 50 minutes at 120°C. This additional heating of 20 minutes resulted in an increase in the soluble nitrogen of the soil extract of about 1 mgm. in 100 cc. Glucose and K_2HPO_4 were added to the soil extract, and the medium was sterilized. As in the previous experiment, it was found to be difficult to maintain a reaction in soil extract favorable for the growth of the nodule bacteria. The pH of the soil extract in this experiment was 7.6 before sterilization and 6.0 after. It was adjusted to pH 6.8 with sterile alkali before inoculation. Five-day-old cultures of the following organisms were used to inoculate the soil extract medium. Alfalfa 100, Red Clover 205 and 202, Pea 310 and 311, and Dalea 901. The cultures were incubated at room temperature, and were shaken on alternate days.

Fifty-three days after inoculation, five flasks of each culture were analyzed for total nitrogen. See table 4 for the results of these analyses, and also of those made when the cultures were 60 days old. For the loss of 0.3 to 0.4 mgm. of nitrogen by the Alfalfa 100 cultures no explanation is offered. No fixation in amounts beyond experimental error is shown by any of these cultures.

Experiment 4

The inconstancy of the reaction of the media in previous experiments made it seem probable that insufficient growth of the bacteria had taken place to give an appreciable fixation of nitrogen More K_2HPO_4 was then used in this experiment than had been used previously; the amount was raised from 0.05 per cent to 0.1 per cent and in addition about 5 gm. of $CaCO_3$ added to one-half of the flasks. Sucrose, because it is much more stable to alkali, was substituted for glucose. The pH of this soil extract medium at the beginning was 7.4 and this was unchanged by sterilization. The addition of 5 cc. of 0.1 *N* HCl caused the reaction to drop to only 6.4. The medium is thus

TABLE 4

The total nitrogen, the percentage of glucose fermented, and the reaction of the cultures
(Fourth experiment)

ORGANISM	NITROGEN ANALYSES AFTER 53 days	NITROGEN ANALYSES AFTER 60 days	GLUCOSE FERMENTED*	REACTION
	mgm. in 100 cc.	*mgm. in 100 cc.*	*per cent*	*pH*
Control	3.9	3.8		5.2
	3.8	3.7		
	3.8	3.7		
	3.8	3.9		
	3.8	Av. 3.8		
	Av. 3.8			
Red Clover 205	3.9	3.8	23.8	4.4
	3.8	3.8		
	4.0	3.7		
	4.0	Av. 3.8		
	4.0			
	Av. 3.9			
Red Clover 202	3.8	3.7	16.8	4.4
	3.8	3.9		
	3.8	3.8		
	3.8	Av. 3.8		
	Av. 3.8			
Alfalfa 100	3.8	3.7	20.8	5.2
	3.2	3.2		
	3.8	3.4		
	3.2	Av. 3.4		
	3.3			
	Av. 3.5			
Pea 310	4.0	3.9	25.3	4.2
	4.2	3.8		
	4.4	Av. 3.9		
	3.8			
	Av. 4.1			
Pea 311	3.8		10.4	4.6
	3.8			
	3.9			
	Av. 3.8			
Dalea 901	4.1	4.0	12.5	4.6
	4.3	4.0		
	4.3	Av. 4.0		
	4.1			
	4.1			
	Av. 4.2			

* Sugar and pH determinations after 35 days.

buffered sufficiently to maintain a reaction favorable for the growth of the root nodule bacteria, even though considerable acid is produced. Each of the following cultures were grown both with and without $CaCO_3$: Pea 310, Alfalfa 100, Red Clover 205, and Soybean 504. The flasks were inoculated with 6-day-old cultures.

TABLE 5

The total nitrogen according to two methods of analysis, percentage of sugar fermented and presence or absence of nitrates in cultures

(Fourth experiment)

ORGANISM	TOTAL NITROGEN		NITRATES	GLUCOSE FERMENTED
	Davisson-Parsons	Gunning modified		
	mgm. in 100 cc.	*mgm. in 100 cc.*		*per cent*
Without calcium carbonate				
Control	4.8, 4.8, 4.7, 4.7, 4.8, 4.9, Av. *4.8*	4.3, 4.3, 4.3, 4.2, Av. *4.3*	+	—
Red Clover 205	4.8, 4.8, 4.7, 4.7, 4.6, Av. *4.7**	5.1, 5.2, 5.2, 5.2, 5.6, Av. *5.3**	—	25.7
Pea 310	4.8, 4.9, 4.9, 5.3, 5.1, Av. *5.0**	5.3, 5.6, 5.8, 5.7, 5.7, Av. *5.6**	—	36.0
Alfalfa 100	4.1, 4.2, 4.1, 4.1, Av. *4.1*	4.2, 4.1, 4.1, 4.1, 4.0, Av. *4.1*	—	12.9
Soybean 504	4.2, 4.7, 4.3, 4.2, 4.3, Av. *4.3*	4.2, 4.1, 4.2, 4.1, 4.4, Av. *4.2*	—	None
With calcium carbonate				
Control	4.7, 4.7, 4.8, 4.2, Av. *4.6*	4.8, 4.1, 4.0, 4.1, Av. *4.3*	+	—
Red Clover 205	5.0, 4.9, 4.9, Av. *4.9*	5.0, 5.4, 5.5, 5.6, Av. *5.4*	—	12.1
Pea 310	5.1, 5.2, 4.7, Av. *5.0*	5.6, 5.5, 5.6, Av. *5.6*	—	29.8
Alfalfa 100	4.5, 4.1, 5.1, 4.5, Av. *4.6*	4.4, 4.2, 4.1, 4.7, Av. *4.4*	+	None
Soybean 504	4.2, 4.8, Av. *4.5*	3.9, 4.3, 4.2, 4.1, Av. *4.1*	+	1.6

* Red clover and pea cultures without $CaCO_3$ developed an acid reaction, pH 6.0 and 6.6.

According to Nobbe and Hiltner (39) it is the bacteroid-form which fixes the nitrogen in the nodules of leguminous plants. If this is the case, bacteroids might be expected to fix nitrogen outside of the host plant. In stained mounts from our cultures, branched forms were observed in the pea, alfalfa, and red clover cultures, and small rods with two deeply staining bodies in the soybean cultures after 14 days of incubation. After 23 days incubation, branched forms were observed in all four cultures, with and without $CaCO_3$.

Sugar analyses, pH readings, and nitrate tests were made on 32 to 34-day-old cultures. These results are given in table 5. The cultures were very irregular in their growth. The alfalfa culture without calcium carbonate had fermented very little sugar (13 per cent), whereas the culture with calcium carbonate did not show any loss of sugar. The soybean cultures with no calcium carbonate, likewise did not show any loss of sugar. Although growth was evident in these cultures no appreciable quantity of sugar was fermented. The analyses on these cultures are included as further checks on the controls.

Five flasks from each series were analyzed for total nitrogen by the Davisson-Parsons method when the cultures were 25 to 26 days old, and the same number at the age of 52 days were analyzed by the Gunning method modified to include nitrates. These results are given in table 5. The cultures analyzed by the Davisson-Parsons method again show no fixation beyond the limit of experimental error. Alfalfa 100 and Soybean 504 cultures appear to have

TABLE 6

Nitrogen analyses of cultures 59 days old with colloidal soil added

(Fifth experiment)

ORGANISM	FLASK 1	FLASK 2	FLASK 3	FLASK 4	FLASK 5	FLASK 6	FLASK 7	AVERAGES
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
Controls	4.8	4.8	4.8	4.9	4.8	4.9	4.8	4.8
Pea 310	5.0	5.1	5.1	5.0	4.9	4.9	...	5.0
Red Clover 205	5.1	4.7	4.7	4.8	4.8	Lost	...	4.8
Soybean 504	4.3	Lost	5.0	4.3	4.3	Lost	...	4.5
Alfalfa 100	4.0	4.1	3.8	3.9	4.2	3.8	...	4.0

lost nitrogen. In the series without $CaCO_3$, the modified Gunning method analyses, on the other hand, show a fixation of nitrogen by Red Clover 205 (1.2 mgm. in 100 cc.) and Pea 310 (1.5 mgm. in 100 cc.) Alfalfa 100 and Soybean 504 show no gain in nitrogen, but check with the controls. The series to which $CaCO_3$ had been added, yielded similar results. It is evident then that the method of analysis is very important. In one of the papers, the modified Gunning method has been used for *culture solutions containing nitrates*, and fixation reported. These results are thus invalid.

Experiment 5

A soil extract was made up like that in the previous experiment (0.1 per cent K_2HPO_4 and 1 per cent sucrose). A colloidal suspension of soil was made to test the effect of colloids upon the nodule bacteria. One gram of water-extracted, dried soil was ground to 100 mesh, suspended in 50 cc. of water and run through a rotary lower plate type colloid mill. One cubic centimeter of this colloidal suspension was added to each flask containing 100 cc. of soil

extract. The flasks were plugged, sterilized, and inoculated in the same way as before, Red Clover 205, Pea 310, Soybean 504, and Alfalfa 100 being used for the experiment. The cultures were incubated at room temperature; 5 days after inoculation, stained mounts were made from representative flasks. Branched forms were observed in all the mounts.

Fifty-nine days after inoculation, analyses of the cultures were made by the Davisson-Parsons method. Table 6 gives the results. No fixation of nitrogen had taken place. In fact, cultures of Soybean 504 and Alfalfa 100 again showed a loss in nitrogen.

SUMMARY

Pure cultures of Alfalfa 100, Bean 402, Dalea 901, Pea 302, 310, 311, and 317, Red Clover 202 and 205, Soybean 501 and 504, and Sweet Clover 115 were grown in a soil extract containing 1 per cent sugar (glucose or sucrose) and 0.05 to 0.1 per cent K_2HPO_4. From 5.4 to 36.0 per cent of the sugar was destroyed under the conditions of these experiments. The cultures were all tested for purity by the litmus milk and potato tests, and all impure cultures discarded. Soil extract cultures when analyzed by the Gunning method modified to include nitrates showed a fixation of as high as 2.5 mgm. of nitrogen to 100 cc. Analyses made of parallel flasks by the Davisson-Parsons method, which is more reliable for the determination of total nitrogen to include nitrate nitrogen, gave evidence of no fixation in amounts beyond the limit of experimental error. Alfalfa 100 and Soybean 501 and 504 consistently gave a loss of 0.1 to 1.7 mgm. of nitrogen to 100 cc.

From more than 500 total nitrogen analyses, it is obvious that nitrogen is not fixed by any of these 12 different cultures of Rhizobia under the conditions prevailing in these experiments.

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NITRATE CHANGES IN A FERTILE SOIL AS INFLUENCED BY SODIUM NITRATE AND AMMONIUM SULFATE[1]

NANDOR PORGES[2]

Rutgers University

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INTRODUCTION

The changes that nitrogen salts undergo when added to soil have attracted the attention of many investigators, and the following workers, Lipman and Blair (5), Coleman (1), Russell (8), and others, have presented extensive reviews on the subject. Nitrate as a form of nitrogen for the plant is very efficient (11), and other nitrogenous compounds are converted to nitrates as a result of microbial activities (9). These activities are influenced by many factors, including temperature (4), moisture (6), aeration (3), soil reaction (12), and energy source (10).

Previous investigations have been conducted with a substrate consisting of sand, or of soil in need of nitrogen. For the experiment herein reported, however, a virgin Sassafras loam having a nitrogen content of 100 mgm. of nitrogen to 100 gm. of soil, and therefore showing no deficiency of nitrogen was employed. Varying amounts of $NaNO_3$ or $(NH_4)_2SO_4$ were added, and the nitrates were determined periodically, by the phenoldisulfonic acid method, the results being presented on the oven-dry basis. All analyses were run on a fresh sample of soil.

EXPERIMENTAL

Accumulation of nitrates in incubated soils

Tumblers each containing 100 gm. of soil were treated in duplicate as follows: (*a*) soil left untreated; (*b*) 2.5 mgm. of nitrogen added as $NaNO_3$; and (*c*) 2.5 mgm. of nitrogen added as $(NH_4)_2SO_4$. These salts were applied from a standard solution, then distilled water was added to the optimum moisture content, which was maintained by the addition of a few drops of

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water whenever necessary. The soils were incubated at a temperature of 27 to 30°C.

Periodically, the soil in each tumbler was thoroughly mixed, weighed, and an aliquot portion equivalent to 10 gm. of dry soil was removed for a nitrate determination. The results are given in table 1.

Although the incubation has increased the amounts of nitrate in the soil, it is noticeable that the treated soils show an increase in excess of the nitrogen added at only the 63-day, 119-day, and 133-day periods. The remainder of the analyses show a diminution of the nitrates. The values shown in table 1 are obtained by deducting the NO_3-N found in the untreated soils from the amounts found in the nitrogen-treated soils, and then noting whether the differences are larger or smaller than 2.5 mgm. (the amount of nitrogen originally added).

The total NO_3-N accumulation and the increase and decrease of the NO_3-N in comparison to the added nitrogen are shown in figure 1.

TABLE 1

Nitrate-nitrogen accumulating in incubated soils

TREATMENT	NITRATE-NITROGEN ACCUMULATED IN 100 GM. SOIL IN TIME INDICATED									
	0 day	21 days	35 days	49 days	63 days	77 days	91 days	105 days	119 days	133 days
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Untreated soil	2.0	3.4	4.2	5.0	6.3	4.9	8.3	8.4	7.2	6.4
2.5 mgm. N added as $NaNO_3$	4.5	5.8	6.3	5.1	10.3	6.4	9.0	9.8	11.8	9.7
2.5 mgm. N added as $(NH_4)_2SO_4$	2.0	5.2	6.0	5.9	9.0	7.4	8.7	10.0	12.7	9.7

Nitrates leached

A series of lysimeters with a capacity for 1000 gm. of soil were prepared from glazed pots, each of which had an opening in the bottom. Into each opening was fitted a one-hole stopper carrying a glass tube to which was attached a piece of rubber tubing. These pots were then placed on a bench, through which the glass tubes penetrated.

For each treatment four pots of soil were prepared for a greenhouse experiment. Two of these pots were left fallow, and in each of the other two, six grains of oats were planted. Distilled water was added whenever it was necessary, to maintain optimum moisture conditions. At the periods indicated in table 2 and 3 the soils were leached with distilled water until the presence of nitrates was no longer detected by means of diphenylamine, and the NO_3-N was then determined in the leachings.

Table 2 shows that a part of the NO_3-N originally present in the soil and also a part of the added nitrogen have been changed to a form not readily

leached from the soil. This "fixed" nitrogen is later recovered as nitrates in the periodical leachings. Although a storing-up or a conversion of the nitrogen occurs, the addition of the nitrogenous salts neither inhibits nor accelerates the formation of nitrates from the original soil nitrogen. From the untreated soil 12.6 mgm. of NO_3-N were leached in excess of the amount present (20 mgm.) at the beginning of the experiment, and values approximating this are

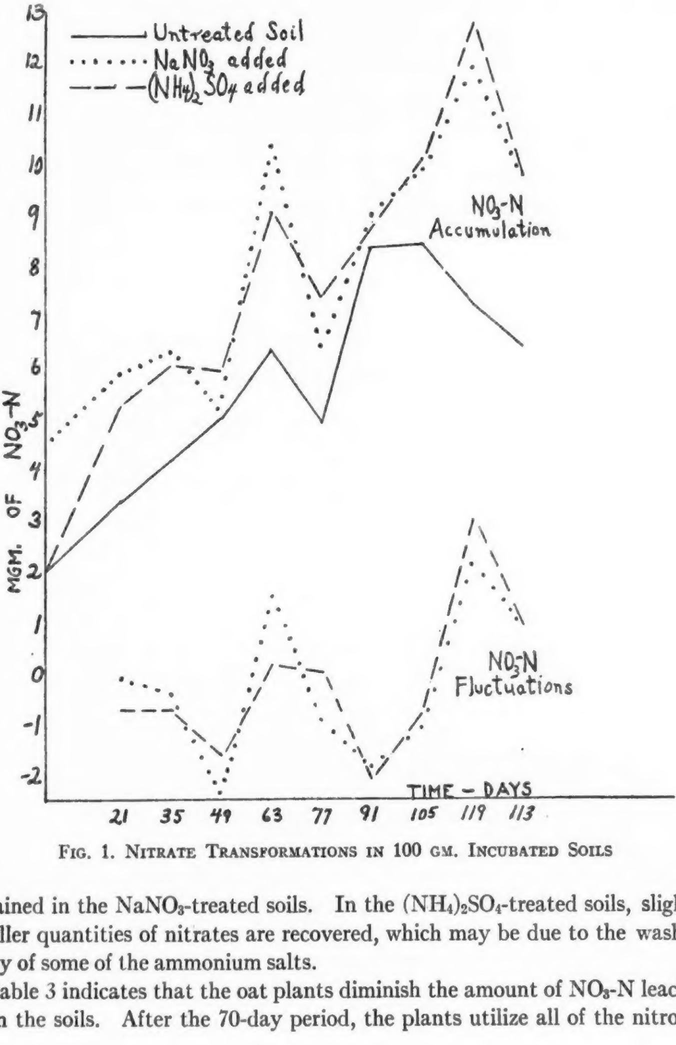

FIG. 1. NITRATE TRANSFORMATIONS IN 100 GM. INCUBATED SOILS

obtained in the $NaNO_3$-treated soils. In the $(NH_4)_2SO_4$-treated soils, slightly smaller quantities of nitrates are recovered, which may be due to the washing away of some of the ammonium salts.

Table 3 indicates that the oat plants diminish the amount of NO_3-N leached from the soils. After the 70-day period, the plants utilize all of the nitrogen

made available; however, this was not sufficient for proper plant development, as was indicated by their growth.

TABLE 2

Nitrate-nitrogen leached from soil kept fallow

TREATMENT	NITRATE-NITROGEN LEACHED FROM 1,000 GM. SOIL IN TIME INDICATED									
	14 days	28 days	42 days	56 days	70 days	84 days	98 days	112 days	126 days	Total leached
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Untreated soil	5.7	1.7	2.9	3.3	3.2	3.0	4.9	3.9	4.0	32.6
Soil + 25 mgm. N as $NaNO_3$	23.0	7.0	5.7	3.6	4.0	3.7	3.3	5.1	2.6	58.0
Soil + 25 mgm. N as $(NH_4)_2SO_4$	16.4	7.1	4.6	3.7	4.1	2.9	3.0	4.8	2.8	49.4
Soil + 50 mgm. N as $NaNO_3$	40.1	11.8	7.8	3.5	4.9	3.5	3.2	3.7	3.5	82.0
Soil + 50 mgm. N as $(NH_4)_2SO_4$	23.2	17.5	13.1	5.6	4.0	5.3	4.7	4.5	2.8	80.7

TABLE 3

Nitrate-nitrogen leached from soil planted to oats

TREATMENT	NITRATE-NITROGEN LEACHED FROM 1,000 GM. SOIL IN TIME INDICATED									
	14 days	28 days	42 days	56 days	70 days	84 days	98 days	112 days	126 days	Total leached
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Untreated soil	4.3	0.3	0.1	0.1	tr.	tr.	tr.	tr.	tr.	4.8
Soil + 25 mgm. N as $NaNO_3$	13.6	1.6	0.1	0.1	tr.	tr.	tr.	tr.	tr.	15.2
Soil + 25 mgm. N as $(NH_4)_2SO_4$	12.8	1.2	0.1	0.1	tr.	tr.	tr.	tr.	tr.	14.2
Soil + 50 mgm. N as $NaNO_3$	26.8	1.8	0.1	0.1	tr.	tr.	tr.	tr.	tr.	28.8
Soil + 50 mgm. N as $(NH_4)_2SO_4$	17.6	6.5	0.3	0.1	tr.	tr.	tr.	tr.	tr.	24.5

TABLE 4

Nitrogen recovered in plants and NO_3-N not recovered from 1,000 gm. soil

	UNTREATED SOILS	25 MGM. N AS $NaNO_3$	25 MGM. N AS $(NH_4)_2SO_4$	50 MGM. N AS $NaNO_3$	59 MGM. N AS $(NH_4)_2SO_4$
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
N in plants	20.4	24.9	23.9	29.9	31.2
N rendered insoluble	27.7	42.4	35.0	53.2	56.1
N not accounted for	7.3	17.5	11.1	23.3	24.9

At maturity (126 days) the entire plants were analyzed for nitrogen by the Kjeldahl method. The amount of nitrogen thus found in the plants and the amounts rendered insoluble in the planted pots are presented in table 4. This insoluble nitrogen is the amount of NO_3-N leached from the planted pots,

TABLE 5

NO_3-N in 1,000 gm. fallow soil and soil planted to oats

TREATMENT	28 DAYS	56 DAYS	84 DAYS
	mgm.	*mgm.*	*mgm.*
Untreated soil:			
Fallow	43.5	35.7	44.0
Planted	22.1	15.5	11.8
25 mgm. NO_3-N:			
Fallow	61.0	52.6	71.6
Planted	39.8	22.4	27.8
25 mgm. NH_3-N:			
Fallow	23.0	55.0	71.0
Planted	22.1	15.0	20.0
50 mgm. NO_3-N:			
Fallow	67.2	88.9	105.8
Planted	63.8	47.0	53.9
50 mgm. NH_3-N:			
Fallow	49.2	55.5	99.0
Planted	32.9	30.1	30.8

TABLE 6

Nitrogen recovered in plants

TREATMENT	28 DAYS	56 DAYS	84 DAYS
	mgm.	*mgm.*	*mgm.*
Untreated soils	21.2	43.7	44.5
25 mgm. NO_3-N	24.0	44.6	47.3
25 mgm. NH_3-N	26.6	46.3	46.9
50 mgm. NO_3-N	24.5	49.3	50.0
50 mgm. NH_3-N	22.6	52.2	52.6

subtracted from the NO_3-N leached from the fallow pots undergoing a similar treatment.

Although additions of the nitrogenous salts have enhanced the plant growth, yet amounts varying from 22.5 per cent to 30.4 per cent cannot be accounted for in the crop and in the leachings. This part that is not readily leached may have been assimilated by soil microörganisms which have derived their energy from the plant residues in the soil. However, plate counts showed practically no difference in numbers of organisms found in the fallow and in the planted soils.

Nitrates in soils not leached

As the soils in the previous experiment had to undergo a severe treatment every two weeks, another series of pots were conducted under more normal conditions. Five series of pots were prepared and the fertilizers were added as in the previous experiment. For each treatment, there were nine pots, three of which were left fallow and six were cropped to oats. At intervals of 28 days, two pots containig plants and one pot containing fallow soil were removed from each series for analyses. Nitrates were immediately determined on a ten-gram sample of soil, and are reported in table 5 on the basis of 1,000 gm. Total nitrogen in the entire plant is recorded in table 6.

The results obtained from the fallow soils again indicate that a proportion of the added nitrogen has been changed to some form other than NO_3-N at the beginning of this experiment, and becomes slowly available, until finally there is recovered a greater amount of nitrogen than was originally added as a fertilizer. The added salts have caused some of the combined soil nitrogen to become available. A greater effect was noticeable with $NaNO_3$ than with $(NH_4)_2SO_4$.

That the plants did not utilize all of the NO_3-N is readily noted, but the addition of the nitrogen fertilizers has increased the growth of the plant to a slight degree and greater growth is obtained with increments of nitrogen. The nitrogen recovery within the plant does not account for all of the NO_3-N that has been changed.

DISCUSSION

A part of the soluble nitrogen is very rapidly changed to an insoluble form, and cannot be determined by the methods used for analysis. Plants growing upon the soil increase this "unaccounted for" nitrogen, but this increase cannot be attributed wholly to microbial activities because the numbers of microorganisms show no marked variation, and neither does the total nitrogen in the soil present marked differences. Hence another causal agent must be sought.

It is possible that the nitrate radical forms a direct chemical or physical union with the soil complexes, the plant acting in some manner as a stimulatory agent. These soil complexes, being in a colloidal form, are able to unite with the nitrate radical (2), forming compounds similar to the oxychlorides (7). As the pH of the soils used varied from 5.5 to 6.2, the conditions were favorable for such a combination.

SUMMARY

1. Fertile soils treated with $NaNO_3$ and $(NH_4)_2SO_4$ were kept under varying conditions, and their NO_3-N content was determined.

2. Incubation of a soil increases the total NO_3-N content. This is not a continuous increase but is either more or less than the amount of nitrogen added as a fertilizer.

3. A portion of the nitrogen added in the salt is rapidly changed to some form that is not readily leached.

4. This insoluble nitrogen becomes available at later periods, as is indicated by the leachings from the soil.

5. The nitrogen in the crop and in the leachings is less than the total nitrogen originally available. Of the available nitrogen, 22.5 to 30.4 per cent is retained in the soil.

6. In the soils not undergoing leaching, a similar retention of the nitrogen occurs.

7. The addition of $NaNO_3$ and $(NH_4)_2SO_4$ mineralizes a part of the soil nitrogen.

8. Even on fertile soils, additions of $NaNO_3$ and of $(NH_4)_2SO_4$ influence plant growth.

9. The plant has a depressive influence upon the accumulation of nitrates in the soil.

10. Lysimeters having 1,000-gm. capacity are described.

11. A suggestion is presented to explain the fate of the added nitrogen that has not been recovered in the plant or in the leachings.

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DIURNAL, AVERAGE, AND SEASONAL SOIL TEMPERATURE CHANGES AT DAVIS, CALIFORNIA

ALFRED SMITH[1]

University of California

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During the fall and spring months there appears to be more interest than at other seasons of the year in the fluctuations of air temperature, probably because they may be of sufficient magnitude to damage crops. Maximum and minimum air temperatures, if of sufficient duration, are of considerable importance in their relation to crop development. The mean daily, monthly, and annual air temperatures as given in most reports are usually obtained by averaging the highest and lowest temperatures. Monthly means are the temperatures usually given in climatological tables, but during the past few years a shorter time, such as a week or a 10-day period, has been used occasionally.

Shaw (6) states that, "In so far as the application of meteorological data to agriculture or phenology is concerned, the week is a much more convenient unit of time than the month." The weekly record prepared by the British Meteorological Office is in the form of accumulated temperatures above 42°F. Geiger (4) has shown that the climate of the atmosphere near the soil is of quite different character from that at higher elevations. He clearly shows that the nature of the ground cover is as important a consideration as the topography and other factors. Plants which do not extend any considerable distance above the soil are under different climatic environment than those which grow to greater heights.

Studies of the leaf temperatures of cotton by Eaton (3) indicate that at Sacaton, Arizona, the yields of certain varieties of cotton, such as the Acala, are greater during cool years than during the years with average maximum summer temperatures above 100°F. Another variety, Pima Egyptian, showed less fluctuation in yields and no relationship to the mean maximum temperatures of the summer. Eaton states that, "It appears probable that the differences in the yields of Acala upland cotton in the years of higher and lower summer temperatures as compared to the yields of Pima Egyptian cotton are associated with the differences found in leaf temperatures of the two cottons."

Patton (5) believes that the maximum air temperature of the day is more

[1] Associate professor soil technology and associate soil technologist in the experiment station, University of California, College of Agriculture.

representative than any other present measure when it is desired to determine for the growing season a temperature that is operative in a beneficial or limiting sense. Smith (8) states that, "For the sake of convenience, the months when the mean daily temperature is between 49° and 72° are considered periods of growth for most crops."

The effective temperature is considered to be the difference between the prevailing temperature and a "zero" temperature. This "zero" temperature as stated by Smith is that temperature below which a plant will make no growth.

From the preceding it is evident that some authorities place stress on the maximum or minimum temperatures whereas others believe that the mean temperatures are of more importance.

PLAN OF EXPERIMENT

Air and soil temperatures have been obtained on the deep recent alluvial soils of the Yolo Series at Davis, California, through a period of years. Details of the experiment have been discussed by the author in another paper (7). The plot in which the soil temperatures which are reported herein were obtained has been kept free of any crop since 1923. After the first of May in general there is little rainfall until the winter rainy period commences, which is usually in October. The soil moisture variations throughout the season were naturally greatest in the surface 4 inches. The air temperatures were obtained by means of a thermograph installed in the usual U. S. Weather Bureau type of shelter. Soil temperatures were obtained at depths of $\frac{1}{2}$, 3, 6, 12, 24, and 36 inches by means of electrical resistance thermometers. The temperature from each individual thermometer was automatically recorded every 15 minutes, day and night, in degrees Fahrenheit. The temperature changes occurring each day during certain months in 1925 and 1927 and the factors influencing them have been shown in a previous paper (7).

It is the main purpose of this discussion to consider average temperatures only.

AVERAGE OR MEAN TEMPERATURES

From the mass of data collected, the daily average temperature was obtained: first, a day average, which is the average of all 15-minute readings for a particular thermometer between sunrise and sunset; next, the night average, or the average of all readings between sunset and sunrise. The day average was then multiplied by the number of hours between sunrise and sunset, and the night average multiplied by the number of hours between sunset and sunrise. The sum of these, divided by 24, gives the daily average temperature. It is believed that a more complete interpretation of temperatures and their effects is possible when the temperature condition existing during the daylight hours when plants are manufacturing food materials is segregated from the night periods. This phase of the study will be discussed in another paper.

From the daily averages, the weekly averages were calculated. The weekly averages form the basis of this paper.

TABLE 1

Weekly average temperatures for 1925 period

DATE	AIR	TEMPERATURES AT DIFFERENT DEPTHS OF SOIL					
		½ in.	3 in.	6 in.	12 in.	24 in.	36 in.
	°F.	°F.	°F.	°F.	°F.	°F.	°F.
2/27	50	51	49	49	50	51	51
3/6	54	60	55	54	53	53	53
3/13	44	53	51	50	50	53	54
3/20	53	63	54	52	52	53	53
3/27	61	70	62	59	58	56	55
4/3	49	54	54	54	56	57	58
4/10	53	63	61	58	58	58	58
4/17	61	72	68	65	63	62	61
4/24	52	62	62	60	61	62	62
5/1	59	76	70	68	67	64	63
5/8	64	78	76	74	72	70	67
5/15	57	69	68	68	70	69	69
5/22	51	70	70	68	67	68	67
5/29	66	80	76	75	72	69	68
6/5	60	72	71	70	71	70	70
6/12	67	83	78	77	75	73	71
6/19	71	87	85	82	79	76	73
6/26	80	95	88	87	83	78	76
7/3	74	90	87	87	84	82	80
7/10	73	89	85	85	83	82	80
7/17	80	92	87	88	84	82	80
7/24	77	92	88	89	87	85	82
7/31	74	90	86	87	84	83	82
8/7	74	91	87	89	86	84	84
8/14	70	85	84	86	83	84	83
8/21	69	84	81	84	82	82	82
8/28	70	84	81	83	81	81	81
9/4	67	82	81	83	81	81	81
9/11	63	79	79	81	79	80	80
9/18	63	76	75	79	77	78	78
9/25	65	76	74	77	75	76	77
9/30	60	74	73	77	74	76	77
Average...	63	76	73	73	72	71	71

RESULTS OBTAINED IN 1925

The 1925 period extended from February 20 to September 30, the time of sunrise varying from 4:39 during the middle of June, to 6:01 on September 30.[2] The time of sunset ranged from 5:49 on February 20, to 7:36 during the

[2] The data for sunrise and sunset were obtained from N. R. Taylor, in charge of the Sacramento, California, office of the U. S. Weather Bureau.

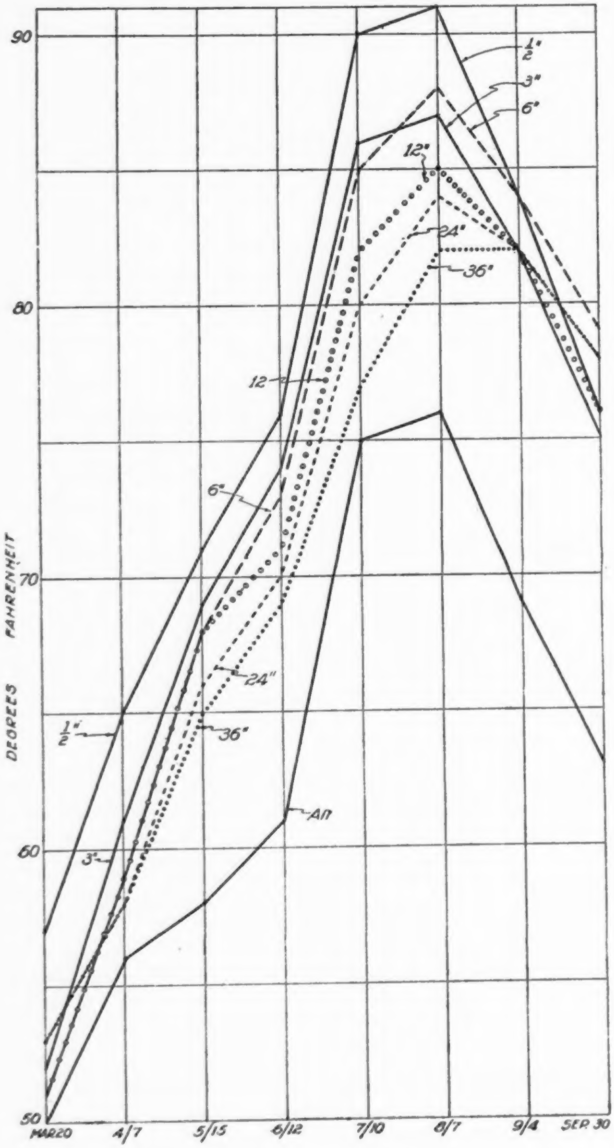

Fig. 1. Average Temperatures by 28-Day Periods for 1925

middle of June. The length of the day was from approximately 12 to 15 hours and the night from 9 to 12 hours. The weekly averages obtained in 1925 are given in table 1, each date mentioned being for the week ending on that date.

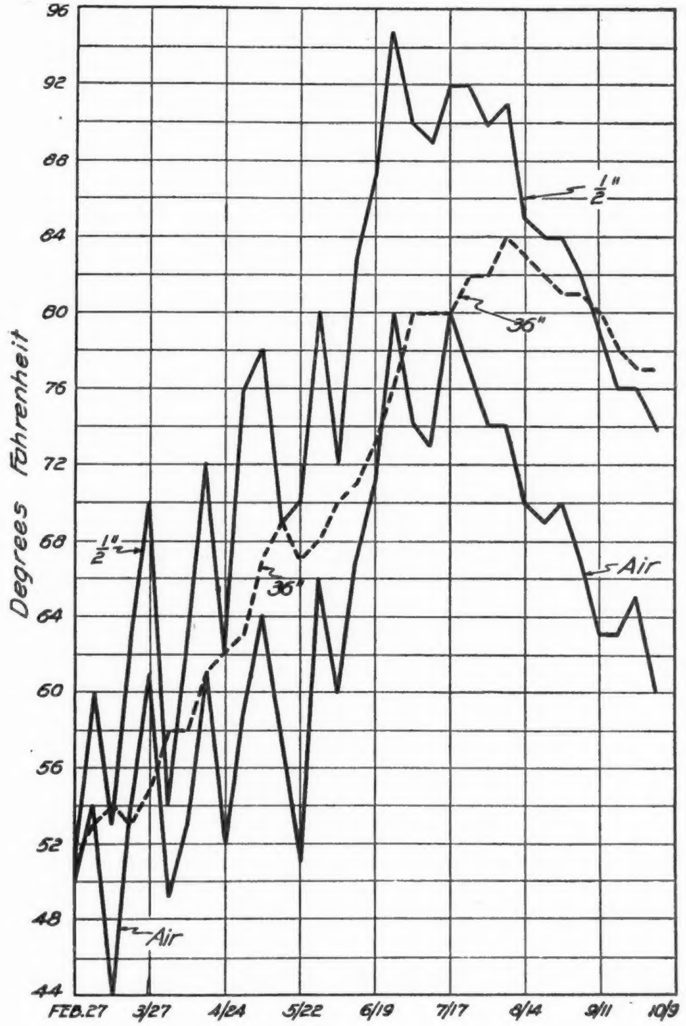

FIG. 2. WEEKLY AVERAGE TEMPERATURES FOR THE AIR, ½- AND 36-INCH DEPTH SOIL TEMPERATURES FOR 1925

The last period, 9/25 to 9/30, is for only 6 days. Because of failures in operating equipment there were a few times, generally not more than 3 hours in extent, when data were not obtained. From the general appearance of the temperature curves it was possible to obtain an approximate average for the day or night period. Air temperatures were obtained at a height of 4½ feet above the soil surface.

An average weekly air temperature of 80° first occurred in the week ending June 26, as shown in table 1. All of the average soil temperatures during this week were from 2 to 8 degrees higher than during the preceding week. During the next two weeks following June 26, the average weekly soil temperatures at the ½-, 3-, and 6-inch depths dropped from 2 to 6 degrees, while at the 24- and 36-inch depths they increased 4 degrees. Average air temperatures of 80° were again obtained in the week ending July 17, when the average weekly temperature at the ½-inch depth was 3 degrees above that of the preceding week. The temperatures at the 3-, 6-, 12-, 24-, and 36-inch depths were from 1 degree to 3 degrees higher during the following week. The average temperature for the entire period for the air was 63°, while the soil temperatures ranged from 76 to 71°. The ranges in the average weekly temperatures were as follows: air, 30°; ½-inch, 43°; 3-inch, 39°; 6-inch, 40°; 12-inch, 37°; 24-inch, 34°; 36-inch, 33.°

Figure 1 summarizes the 1925 data more briefly. It gives the average temperatures by 28-day periods, the first 28-day period ending on March 20. In figure 2 only the weekly average temperatures for the air, ½- and 36-inch depth soil temperatures are shown, as the temperatures for the other soil depths lie, in general, between the ½- and 36-inch depths. In this figure the general parallelism between the air temperature and the soil temperature at the ½-inch depth is well shown, while the curve for the 36-inch depth is much smoother, as would be expected.

In a growing season of 140 days, extending from February 20 to July 10, the average air temperature was at this time 60°, whereas for the ½-inch depth, it was 72°, and for the 36-inch depth it was 64°, or a range in the soil temperatures between these two depths of 8 degrees.

RESULTS OBTAINED IN 1927

During the 1927 period, which extended from January 1 to June 18, the time of sunrise varied from 7:26 on January 1, to 4:39 on June 10 to 18. The time of sunset ranged from 4:54 on January 1, to 7:34 on June 18. The length of both day and night therefore ranged approximately from 9 to 15 hours. The weekly averages are given in table 2, each date given being for the week ending on that date.

The highest average temperatures, as shown in table 2, occurred during the last week. The greatest range in the soil temperatures occurred in the week ending April 30, when at ½-inch it was 77°, and at 24 inches 64°. Beginning with the week ending February 19, to the end of the week of March 12,

a range of only 2 degrees was obtained at the various soil depths. A similar period was from the week ending April 2 to the week ending April 16. The average of the air temperatures for the entire period, January 1 to June 19, was 54°, while the average soil temperatures were practically the same (61°, 60°). The range in the average weekly temperatures were as follows: air, 32°; ½-inch, 40°; 3-inch, 37°; 6-inch, 35°; 12-inch, 34°; 24-inch, 21°; 36-inch, 26°.

TABLE 2

Weekly average temperatures for 1927 period

DATE	AIR	TEMPERATURES AT DIFFERENT DEPTHS OF SOIL					
		½ in.	3 in.	6 in.	12 in.	24 in.	36 in.
	°F.	°F.	°F.	°F.	°F.	°F.	°F.
1/8	48	51	50	50	49	53	51
1/15	45	49	49	49	49	54	52
1/22	43	47	48	49	49	54	52
1/29	43	45	46	47	47	52	50
2/5	48	50	50	51	50	52	51
2/12	46	48	47	49	49	53	52
2/19	51	52	51	51	50	52	51
2/26	54	55	55	55	53	54	53
3/5	48	53	53	54	54	55	54
3/12	50	54	54	55	54	55	55
3/19	49	53	53	54	53	56	55
3/26	54	61	60	58	56	56	55
4/2	52	57	58	58	56	58	58
4/9	51	59	59	59	57	58	58
4/16	50	58	58	58	57	58	58
4/23	58	67	65	65	63	60	61
4/30	62	77	73	73	71	64	67
5/7	58	72	71	71	70	68	69
5/14	62	75	73	73	70	67	69
5/21	64	78	77	77	75	70	73
5/28	61	75	74	73	73	70	72
6/4	64	77	74	74	72	70	71
6/11	67	77	75	75	74	72	73
6/18	75	85	83	82	81	73	76
Average...	54	61	61	61	60	60	60

It should be noted that during the last 3 weeks the average temperature at the 36-inch depth increased 5 degrees, and at the 24-inch depth 3 degrees.

In figure 3 the average temperatures by 28-day periods are shown, the first period ending on January 29. Figure 4 shows the weekly average temperatures for the air, ½-, and 36-inch depths. The parallelism of the air and the ½-inch depth temperatures is again well shown, with the 36-inch depth curve more smooth.

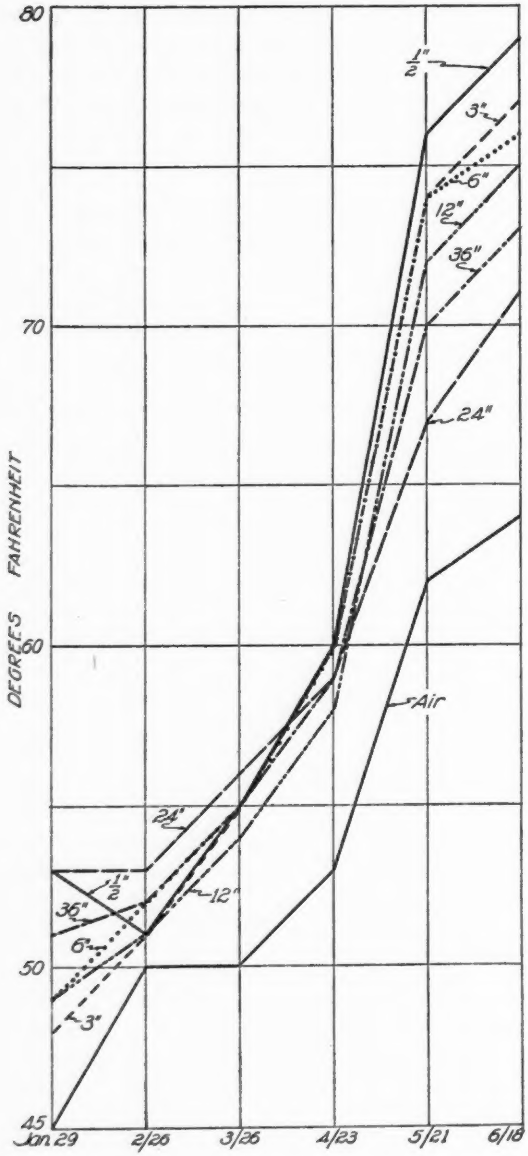

FIG. 3. AVERAGE TEMPERATURES BY 28-DAY PERIODS FOR 1927

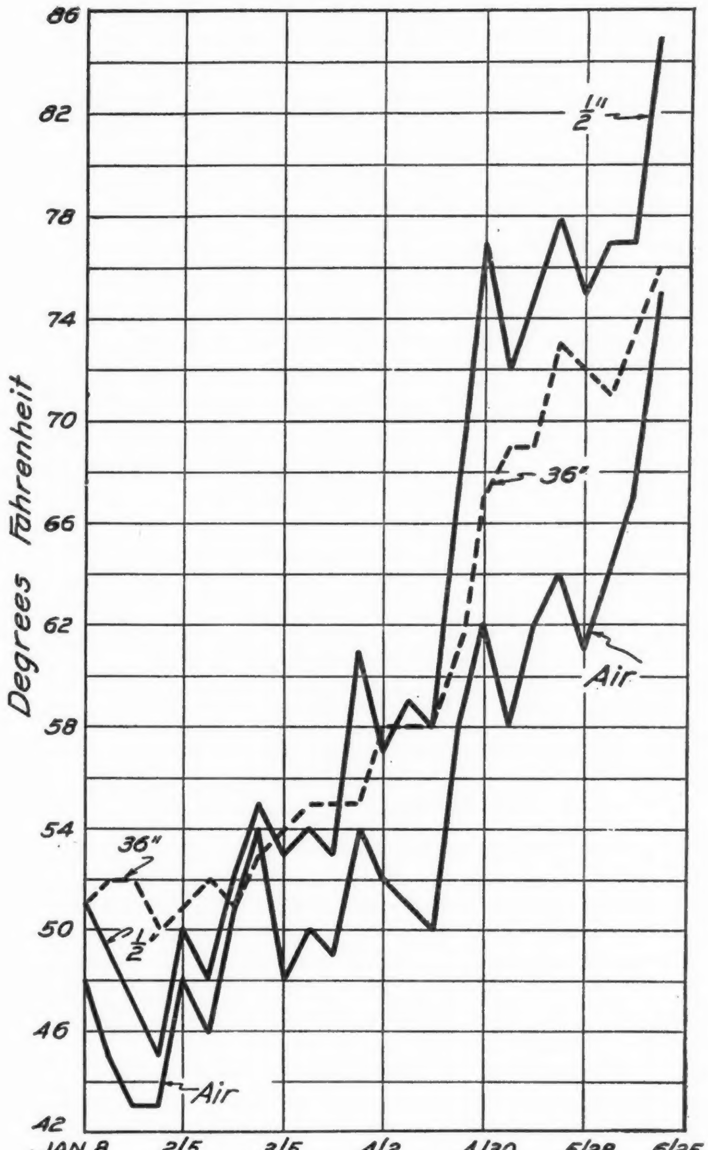

Fig. 4. Weekly Average Temperatures for the Air, ½- and 36-Inch Depths Soil Temperatures for 1927

AIR TEMPERATURES AT MIDDAY COMPARED WITH LATE AFTERNOON

The belief that the air temperatures on summer days at 5 p.m. are higher than those at noon of the same day is borne out by the records of June, 1925, when for this 30-day period the air temperatures at 5 p.m. averaged 1.5 degree higher than those at noon. On any one day, however, the difference may be greater, or the noon readings may be the highest. On a calm, clear day the late afternoon is warmer than at midday. The character of the sky and the velocity and wind direction often change during the day and these naturally affect the comparisons. In June, 1925, on some days the 5 p.m. readings were as much as 8 degrees cooler and on other days 10 degrees warmer than the noon readings. The noon readings averaged 85.9° for the month.

METHODS OF OBTAINING DAILY TEMPERATURES

Various records have been used to express daily temperature changes. Carter (2) using standard soil thermometers has reported data for depths of 1-inch, 3-, 6-, 9-, 12-, 24-, and 36-inches occurring at Lincoln, Nebraska, by reading the thermometer once daily, at about dark. He found no apparent indication of unusually high or low air temperatures at the 24- and 36-inch depths.

Bouyoucos (1) took temperature readings at 6 a.m., 1 p.m., and 5 p.m. to obtain a daily average, as he believed that these gave "a very close approximation of the true daily average and fluctuation of temperature for all soils and for all depths." He does not claim, however, that they gave "the absolute average and range of temperature for all depths and for all soils." In order to evaluate this method, two periods were taken from the records and the daily average of the temperatures at 6 a.m., 1 p.m., and 5 p.m. were determined and compared with the averages obtained from the 96 daily readings obtained at 15-minute intervals.

Results in June, 1925

During this month the time of sunrise varied from 4:39 to 4:43 and sunset from 7:26 to 7:36. The air minimum occurred about sunrise and the air maximum from four to five hours before sunset. The lag of the maximum and minimum soil temperatures as compared to time of occurrence of the air maximum and minimum was approximately as follows: ½-inch—1 hour, 3-inch—2 hours, 6-inch—4 hours, and 12-inch—8 hours. Data are reported for the ½-, 3-, 6-, and 12-inch depths only, as it is at these depths that distinct maximums and minimums occur at Davis.

With the average of the 15-minute readings as a standard, the average obtained by the three daily readings was found to be 6 degrees higher for the air temperatures, 8 degrees higher for the ½-inch depth, 1 degree higher for the 3-inch depth, identical for the 6-inch depth, and 1 degree less for the 12-inch depth, for the month. Greater differences were found among individual daily readings. On some days the averages of the three daily readings were as much

as 10 degrees higher for the air temperatures, 13 degrees higher for the ½-inch depth, and 2 degrees higher for the 3-inch depth, than the daily (24-hour period) average of the 15-minute readings. For the 6-inch depth they were from 2 degrees less to 2 degrees more, and at the 12-inch depth they were at times 2 degrees less than the averages of the 15-minute readings.

The day (sunrise to sunset) average of the 15-minute readings was always within 4 degrees of the average of the three readings. The night (sunset to sunrise) averages were 31 degrees higher for the air temperatures, 34 degrees higher at the ½-inch depth, 6 degrees higher at the 3-inch depth, 4 degrees lower at the 6-inch depth, and 2 degrees lower at the 12-inch depth, than the corresponding average of the three readings. As the length of the day was nearly 15 hours and the night only about 9, the large differences noted between the night averages and the three readings are considerably reduced when the daily (24-hour) period is considered. The average of the three records obtained at 6 a.m., 1 p.m., and 5 p.m. during the month of June, 1925, therefore was comparable to the average of the 15-minute readings only for the 3-, 6-, and 12-inch depths and not for the air or ½-inch depth when the month is taken as the unit. Daily differences as noted were of greater magnitude for the air and all soil depths.

Results from May 21 to June 20, 1927

A similar comparison was made for the period of May 21 to June 20, 1927. During this time sunrise varied from 4:40 to 4:48 and sunset from 7:17 to 7:34. The occurrence of the air minimum and maximum with respect to sunrise and sunset and the lag of the soil temperatures were practically the same as during June, 1925. Again when the average of the 15-minute readings was used as a standard, the average of the three daily readings was found to be 5 degrees higher for the air temperatures, 5 degrees higher for the ½-inch depth, 1 degree higher for the 3-inch depth, the same for the 6-inch depth, and 1 degree lower for the 12-inch depth for the month.

As in the earlier period, greater differences were found among individual daily readings. On some days the average of the three readings were as much as 10 degrees higher for the air temperatures, 7 degrees higher for the ½-inch depth, 3 degrees higher for the 3-inch depth, and 2 degrees higher for the 6-inch depth than the daily (24-hour period) average of the 15-minute readings. At the 12-inch depth they were from 2 degrees less to 2 degrees more than the 15-minute averages. The day (sunrise to sunset) average of the 15-minute readings was always within 4 degrees of the average of the three daily readings. The night (sunset to sunrise) averages were 29 degrees higher for the air temperatures, 18 degrees higher at the ½-inch depth, 7 degrees higher at the 3-inch depth, from 3 degrees less to 3 degrees more at the 6-inch depth, and 4 degrees less at the 12-inch depth than the average of the three daily readings. The same conclusions relative to the value of three readings as compared to a more complete record of the temperature changes can be drawn for this (1927) period as was done in the 1925 period.

SUMMARY

During the period of February 20 to September 31, 1925, the average air temperature was 63° while the average soil temperatures for the ½-, 3-, 6-, 12-, 24-, and 36-inch depths ranged from 76° to 71°. The ranges in the average weekly temperatures were for the air, 30°; ½-inch, 43°; 3-inch, 39°; 6-inch, 40°; 12-inch, 37°; 24-inch, 34°; and 36-inch, 33°.

In the second period, January 1 to June 18, 1927, during certain weeks the average soil temperatures for the ½-, 3-, 6-, 12-, 24-, and 36-inch depths were within 2 degrees for all depths. The average air temperature in the 1927 period was 54°, whereas the average soil temperatures were practically the same (61°, 60°) for all depths. The ranges in the average weekly temperatures were for the air, 32°; ½-inch, 40°; 3-inch, 37°; 6-inch, 35°; 12-inch, 34°; 24-inch, 21°; and 36-inch, 26°. The importance of the week as a phenological unit and in the determination of effective temperatures is emphasized. The average temperatures for the air, ½-, and 36-inch depths are shown graphically for both the 1925 and 1927 periods. The parallelism of the air and the ½-inch soil depth temperatures is well shown and the data of the soil temperatures at a depth of 36 inches produce a smoother curve.

A comparison of the midday and late (5:00 p.m.) afternoon air temperatures was made to show that during a period such as a month, the differences are slight (1.5 degrees higher at noon) but that on certain days the 5 p.m. readings were as much as 8 degrees cooler and on other days 10 degrees warmer than the noon readings.

Daily average temperatures obtained by using three readings daily (6 a.m., 1 p.m., and 5 p.m.) were compared with the average obtained by using 15-minute readings, or 96 in a 24-hour period. Two warm periods were selected, one in 1925 and the other in 1927. The average of the three readings was comparable to the average of the 15-minute readings only for the 3-, 6-, and 12-inch depths and not for the air or ½-inch depth when the period taken was one month. Daily differences between the averages obtained in the two ways noted are of considerable magnitude. The day (sunrise to sunset) average of the 15-minute readings was always within 4 degrees of the average of the three readings.

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SOIL PROFILE STUDIES: II. METHODS USED IN THE PROFILE SURVEY OF NEW JERSEY SOILS

J. S. JOFFE* AND L. L. LEE

New Jersey Agricultural Experiment Station

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Until recently the study of soils in the United States, England, and Western Europe was primarily limited to the surface, sub-surface, and subsoil. Such an approach to the study of soils was prompted by the agronomic point of view; the underlying motive was to discover the facts responsible for this or that behavior of the soil mass in relation to plant growth. The title, and in a large degree the content, of the valuable contribution of the eminent British soil scientist, Sir John Russell (7) "Soil Conditions and Plant Growth" typify the aforesaid. Little consideration has been given to the study of soils as such. In truth, soil studies dealt with soil material, not with soil—an independent natural body—the features and characters of which may be studied only by analyzing its anatomy as exposed in a cross-sectional, or profile, cut.

The voluminous data of the soil survey in New Jersey, and for that matter all over the United States, accompanied by the chemical and physical analysis of the soils, enriched our store of knowledge of the soil material, its geological and mineralogical origin, and its mode of formation and composition. It failed, however, to disclose the facts of the internal make-up and construction of the soil as a natural body or object; it failed entirely to follow the upward and downward movement and the translocation of the ingredients of the soil mass and their decomposition products through the soil body.

The discoveries made by the Russian scientists, discussed in a former paper (2), taught us that the soil in its anatomical make-up is composed of distinct layers, or horizons, genetically related, expressing definite chemical, physical, and biological characteristics and features. In the light of these, the forces responsible for their formation, or the soil-forming processes, become apparent. The characteristics and features of the horizons indicate the probable tendencies in the soil-forming processes and thus enable us to treat the soil in such a manner as to retain the desirable features and to maintain the conditions best suited for the ultimate aim of our studies—the productivity of the soil.

It is the study of the soil horizons in the soil profile with "no reference to other considerations," as expressed so forcibly by Marbut (6), that will help to unravel the mysteries of the "soil conditions in relation to plant growth."

*In paper I of this series, appearing in the July number of SOIL SCIENCE, the following footnote was omitted from p. 46, l. 24: "V. V. Poluinov has presented a paper on this subject, 'Contributions of Russian Scientists to Paleopedology'—Academy of Science, Leningrad, 1927, p. 1-33."

These brief considerations will suffice to introduce the subject of the profile studies of New Jersey soils. With the accumulation of the data on the physical, chemical, and perhaps biological properties of the soil material from the profiles investigated, the various relationships manifesting themselves within the soil profile will be discussed and analyzed. This, however, is to be preceded by a presentation of the methods used in the investigations. Because of the scantiness of material reported in the English literature on methods of soil profile studies—save that of Marbut's outline (6), which is excellent for a general orientation—it was deemed advisable to present the methods somewhat in detail.

METHODS

Choice of location

The accumulated knowledge on the soils of the state in the hands of the Soil Survey and the system of the Survey in arranging the soils into definite series and types[1] were utilized in determining to a certain extent the locality for the profile studies. The underlying motive in this respect was an attempt to correlate the already known facts with those sought by the comprehensive study of the profile, which is an aim by itself. The most important soil series and characteristic types within them were kept in mind, while, in general, the choice of the locality was determined primarily by the principles underlying the genetic point of view of soils.

Well-developed typical soil individuals were selected on well-drained areas, free from undulations due to the microrelief. Preference was given to virgin soils, since cultivation destroys the natural constitution of the surface horizon or horizons, thereby influencing the lower horizons and masking the nature of the effects of the soil-forming processes. Observations and data obtained on virgin soil profiles lend themselves to interpretations, which may be then applied to tilled soils of the same genetico-morphological nature. Studies on tilled soils will follow the profile survey of the virgin soils.

A preliminary study of the Soil Survey maps and reports was made as an orientation of the geography, topography, geology, and, to some extent, the climatic conditions of the area in which the profile cut or cuts were made. For future reference, the particular spot has been marked on the reference map.

Digging the soil profile cut

A trench 2.5 to 3 feet wide and as deep as required to reach into the parent material was dug for each profile. The length of the trench was determined by the depth required. Both sides and one end of the trench were cut down to form vertical walls. The other end was dug down to a slope with a step-like arrangement. This makes the trench easily accessible and affords a comfort-

[1] "Type" in the sense of a textural unit and not in the sense used by the Russian school of soil science, which indicates a certain soil-forming process, such as podzol "type" and chernozem "type."

able seat while observations and notes are being made. These steps also serve as a support for the worker when the spade is forced horizontally into the opposite end of the trench along the plane of the horizons to dig out a block of soil from any particular horizon for sampling.

In laying out the trenches to be dug, two important things were considered: First, the position of the trench was marked off on an area visibly free from heavy roots,[2] especially toward the vertical end of the trench. Heavy roots traversing the path of the exposed cut at that end obscure somewhat the features of the horizons and make it difficult for sampling. Second, the same end of the profile should be exposed so as to insure proper light effects (direct sunlight is to be avoided) for the differentiation of the color changes in the horizons.

The soil material dug out is thrown over to one side away from the edge of the trench; the dead leaves and wood are removed from the edges of the trench leaving the leaf-mold layer exposed.

Examination of the morphological features

The procedure followed in the examination of the soil profile was the one practiced by the leading Russian soil morphologists, with whom the authors were in contact while on the transcontinental tour with the First International Congress of Soil Science [1927],[3] and described in the voluminous Russian literature, cited in a former publication (2). A number of terms used by soil workers in the English-speaking countries in designating certain characteristics of soil material have been substituted and included in the descriptions used in the procedure. Thus, for instance, the excellent term "texture" has been substituted for "soil skeleton," which is used by the Russians.

The successive steps of the procedure may be presented, following the scheme of Zakharov (8), in outline form as follows:

1. Constitution: compactness and consistency of the soil.
2. Habitus of the profile.
3. Depth of profile and thickness of respective horizons.
4. Texture of soil material.
5. Color of soil.
6. Structure
7. Concretions, foreign intrusions.
8. Miscellaneous observations.

The methods of approach in studying each one of the enumerated soil profile attributes were as follows:

1. Constitution.—By tapping the soil with gentle downward strokes with a sharp, rounded, shallow hand scoop or similar instrument along the surface of the wall of the exposed cut, one may readily feel in his hand when the scoop

[2] The virgin soils in New Jersey are almost all covered with woods.

[3] Proceedings and papers of the Fist International Congress of Soil Science, v. 1, published in 1928.

hits a more compact or a looser horizon. After a little practice the various horizons in the soil profile may thus be differentiated and outlined.

The separate horizon established, a closer examination of them is undertaken. One may note the constitutional attributes: porosity, ease of falling apart of the structural soil units when crushed lightly in the palm of the hand, stickiness, plasticity, friability, looseness. In the B horizon of accumulation there is a sharp rise in compactness, a fundamental constitutional character of this horizon, and an increase in plasticity, especially in the humid regions. The alkali soils or the heavy type of chernozem in the semi-arid regions may possess such compacted horizons, developed to a high degree; a pick-axe or crowbar must be used on them. Something on that order of compactness was observed by the authors in examining a soil profile on the alkali soils of Fresno, California. The soils in New Jersey, as a rule, show no such striking constitutional characters, even in the B horizon of the heavy clay soils. The overlying A horizon or subhorizons are usually of a loose or mellow constitution.

The constitution of the soil horizons is of prime importance from the practical standpoint. A soil with a loose constitution is more easily tilled; the constitution of the soil determines to a certain extent the moisture and air régime of the soil.

2. Habitus of the profile.—Having established the different horizons in the profile from the constitution of the soil, one may proceed to describe the habitus, or the general appearance of the profile.

In a virgin forest soil of the temperate climatic zone, like New Jersey, the surface, upon the removal of undecomposed dead leaves, woody material, and stems of dead plants, reveals a mat of humified material intermingled with some mineral soil material. It is usually a thin layer, varying in thickness from 1 to 5 cm. or more. In the regions with a cool climate this surface subhorizon may reach a thickness of 30 cm. or more, giving rise to the so-called "Rohhumus." Zakharov (9, p. 8), Kosovich (4, p. 2), and other Russian investigators call this subhorizon and the underlying subhorizon "the humus accumulative horizon," designated by the letter A. Genetically the soil-forming processes manifest themselves in the accumulation of the decayed organic materials and in the formation of this surface horizon.

In the grass lands, like the steppe or the prairie, this decay (humus)-accumulative horizon is not divided into the subhorizons as in the soils of the temperate climate. The entire surface A horizon blends in with the true eluvial or leached horizon, of which we shall speak presently.

The surface subhorizon of the humus-accumulative horizon in the New Jersey soil profiles studied by the authors was designated as A_0. This horizon is accumulative in another sense: it becomes enriched with mineral substances in the mineralization process of the organic matter, which obtains these substances from the mineral fractions of the horizons below. Part of these mineral substances, as well as the mineral fraction of the intermingled soil material, undergo leaching, and in this respect A_0 is related to the horizon of eluviation.

Part of these movable mineral substances become fixed with the humus, forming the humate complexes.

The horizon immediately below A_0 possesses the humus-accumulative properties to a less degree than A_0. It approaches in this respect the make-up of the horizon of eluviation and suffers the mechanical and chemical reactions of the same horizon. Since it differs from A_0 and is more like the horizon of eluviation, it has been designated, whenever found, as A_1. In the heavier soils the A_1 horizon sometimes overlies the horizon of illuviation (washing in), known as horizon B. Ordinarily, however, it has a distinct eluvial horizon. In the lighter soils the A horizon—from A_0 to B—is usually divided into one, two, or more subhorizons designated as A_1, A_2, A_3, etc. The constitution of the soil, its texture, structure, and color, serve as guides for subdividing the A horizon. The A_2 and A_3 subhorizons usually constitute the pronounced eluviation horizon. This horizon of eluviation is frequently called the "transitory horizon," being genetically developed between the humus-accumulative and the illuviation horizons. The chemical analyses, which are in progress in the laboratories of the experiment station, will check up the divisions made on a morphological basis. The Ca, Fe, Al, and SiO_2 contents in the soil material of the horizons give an unmistakable picture of the genetic relationships.

The B horizon of illuviation is located below the horizon of eluviation. The substances washed out from the overlying horizons by mechanical and chemical forces are in a great measure caught in this horizon. It is easily recognized by its constitution, as has been described. Often one may detect in the B horizon, on the basis of constitution, texture, concretions, and color, certain subdivisions and, in the soils studied, these were marked B_1, B_2, etc.

The parent material below the B horizon is designated as C. This may be rock or unconsolidated material; it is the unweathered or incompletely weathered geologic formation.

In slightly podzolized soils—a condition found in a number of soils examined in New Jersey—the general appearance of the soil profile is not fully expressed. The effects of podzolization are not morphologically apparent, although the chemical analyses unmistakably indicate podzolization.

Zakharov (9) points out that the profile characteristics of soils depend on the character of the soil-forming process. Thus the podzol type of soil formation will manifest a definite profile type and the chernozem type of soil formation, another profile type. Besides these fundamental types, which are enumerated and illustrated in the following, there may be a great variety of types due to the properties of the parent material—its texture, relief, age, and degree of development of the soil.

The fundamental types of profiles based on the types of soil-forming processes are illustrated in figure 1, reproduced from Zakharov (8). These are: 1. Podzolized type; 2. Chernozem; 3. Serozem (gray soil); 4. Alkali; 5. Bog.

3. Depth of profile and thickness of respective horizons. The well-drained soils examined in New Jersey vary in their depth of profile as well as in the thickness

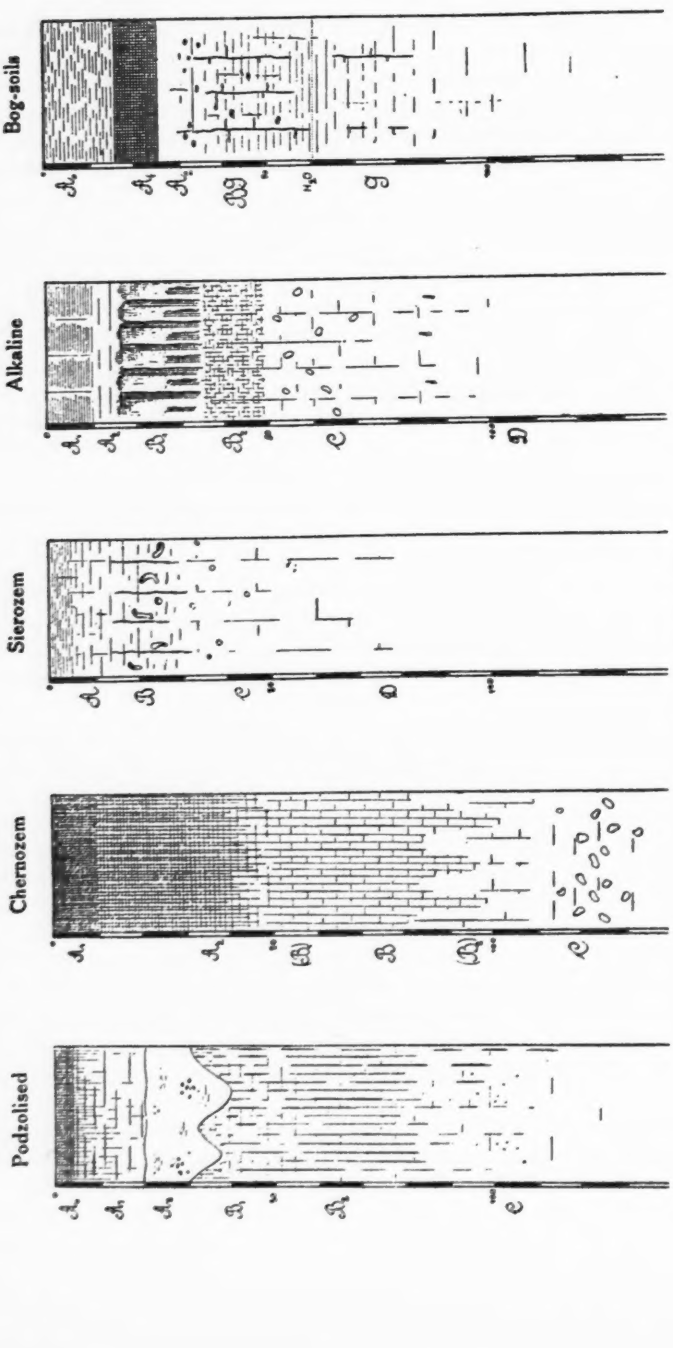

FIG. 1. PROFILE TYPES

of the individual horizons. The A_0 horizon is usually 2 to 5 cm. thick; the A (A_1, A_2, A_3, etc.) horizon varies from 15 to 60 cm., with an average of 35 cm; the B horizon varies from 15 to 40 cm., with an average of 28; the total depth of the profile varies from 50 to 95 cm. (down to horizon C—the parent material). The relation (if any relation does exist) between the soil series as understood by the soil survey and depth of profile will be discussed in the forthcoming detailed studies of the profiles.

4. *Texture of soil material.*—The texture of the soil material serves as an excellent supplementary attribute in determining the constitution of the soil. In itself, the texture of the horizons is a characteristic feature of the genesis of the soil. Usually the upper horizons are of lighter texture than those below. The B horizon of accumulation, where the finer particles are washed in, is of course of the heaviest texture throughout the profile, unless the parent material is a clay.

The comprehensive scheme of the Soil Survey to classify the soil series into various types according to the texture of the surface soil has been applied in the soil profile studies to differentiate the various horizons in the profile.

The field observations on the texture are to be checked in the laboratory by the method of mechanical analyses.

5. *Color of the soil.*—The successive horizons in the profile may be differentiated according to differences in color. In some cases slight color variations may be encountered within one horizon, usually in the subhorizons of eluviation. The distribution of organic matter is usually responsible for such variations. These do not, however, impart to any horizon such properties as would differentiate it as a separate horizon. In general the color of the soil varies within wide limits, depending on the component parts of the soil—one that is rich in organic matter is dark or black in color; one rich in iron is red or yellow depending on the state of oxidation of the iron; one depleted of its organic matter and iron with a residue of SiO_2 shows a whitish-gray color.

Almost all virgin soils have a dark upper horizon, because it is the horizon of organic matter accumulation. The color of the eluvial horizon varies with the type of soil formation; thus the chernozem type is dark, the podzol is grayish-white. The general run of color in this horizon of the 11 different soil types—in the sense of the Soil Survey—investigated in New Jersey is yellowish-brown, with the exception of the Penn series which has an eluvial horizon reddish-brown to red in color. The B horizon as a rule is similar in color to that of the parent material, changing in tone because of the material washed in and incrustations formed.

The horizons of all the soils were examined for color on the freshly exposed cuts, and the soil material from each horizon was later again examined in the laboratory for color in the dry state. Usually the color of the soil material when dry attains a lighter shade.

6. *Structure of the soil.*—The type of aggregation units of soil material or the type of build of the solid portion of the soil is known as the structure. It is the

resultant of the genesis of the soil, the arrangement of the soil ingredients under the influence of the forces of soil formation. The soil structure in its natural state is a constant modified by the electrolyte content of the soil, which in turn is regulated by the weather conditions. Mechanical disturbances, like tillage and chemical treatment such as fertilizer additions, will greatly influence the structure. In recording the structure of the soil we must remember that there is a macro- and micro-structure. The micro-structure makes up the pattern of the mineralogical elements which upon cementation with the colloidal materials build the aggregation units of the macro-structure. It is the macro-structure which we can observe in the soil profile, which gives to the soil its channels, cracks, openings—in other works, its porosity. The porosity units may follow both the vertical and horizontal planes. Through these channels the gravitational waters and gases move freely; even convection currents may function through these openings. The diffusion of gases and the capillary movement of water take place within the units of the micro-structure.

The single-grain structure which indicates practically no micro-structure was noted in the sandy soils studied. In the soils of finer texture, the structure is more complicated. The method followed in designating structure was that of Zakharov (8). He recognizes three fundamental types of structure (macro): I. cubic: three equal axes at right angles to one another, II. prismatic: the vertical axis longer than the horizontal, and III. platy: the horizontal axes longer than the vertical. Within these types one may find the various forms of structure: amorphous, adobe, fluffy, cloddy, nutty, granular, crumbly, columnar, foliated, buck-shot, and different variations of these forms related to the size of the aggregate units.

7. *Concretions and foreign intrusions.*—Concretions of limestone and gypsum are very common in the soils of the less humid regions. These types of concretions are the most common; they are not, however, the only kind found. Iron and manganese concretions are frequently encountered. Depositions, replacements, and precipitation of various chemical substances along the paths of roots give what is known as veins. In some soils we have deposition of chemical substances around the structural units, giving the appearance of mycelium. The underground passages of rodents and other forms of soil-inhabiting life may also become spots of concretions. Besides these, one may find foreign material brought in by water, glaciation, and often by animals and humans. All of that is to be noted in the study of the soil profile.

8. *Miscellaneous observations.*—The depth of the water table, whenever possible, was determined and any effects on the soil due to the upward movement of the water were noted. The so-called gley formations, or mottling effects, are typical illustrations of a high water table.

In the humid belt, like New Jersey, the soils with a well-developed profile have no free carbonates in the upper horizons of accumulation and eluviation, even if the parent material is of limestone origin. On such soils the depths of the carbonate-free horizon were noted. This horizon should not be confused with the horizon of lime carbonate accumulation encountered in regions of low

rainfall: "less than 17 to 18 inches per year in cool climate and 30 inches in hot or very warm climate." [*See* Marbut (6)].

The depth of root penetration of the native vegetation was another point to be noted. The surface native vegetation was recorded. This is important, inasmuch as the mineral constitutents derived from the organic matter influence the reaction and composition of the horizon of accumulation. The percentage of ash of the forest cover from the following species of trees as given by Glinka (1, p. 30) will illustrate the point: spruce—1.46 per cent, pine—4.52 per cent, beech—5.57 per cent, heather—3.09 per cent.

Observations were made on the distribution of organic matter in the soil profile. Such observations give an idea of the movement of the organic matter. The chemical analyses on the organic fraction in the respective horizons will show the nature of the movement and the mode of distribution of the organic matter.

Sampling of the soil profile

The next step, after the features of the soil profile have been recorded, was sampling the soil.

The respective horizons were marked off on the end and side walls of the profile cut. With the aid of a special sampling tube, described by Lebedev (5), samples were taken from the horizons on the side walls for the determination of the moisture and volume-weight of the soil. Plate 1, reproduced from Lebedev's paper, illustrates the method:

The sharp end of the steel tube (pl. 1, fig. 1) is inserted, without turning, into the wall of the soil cut at a depth of 5 to 6 cm. (pl. 1, fig. 2). With the aid of a long narrow knife the soil from the outer end of the tube is cut off at an angle so that there remains a heaping surface above the cutting edge of the tube (pl. 1, fig. 3). The tube is then removed from the soil and the surplus from the inner and outer edge is carefully removed with the sharp edge of the knife. This gives a cylinder of soil with an undisturbed structure.

The soil is placed into a tared aluminum moisture dish, weighed, dried at 100 to 105°C., and again weighed. The loss in weight gives the moisture content; the dry weight of the soil and volume of cylinder known, the volume weight of the soil is determined. Three to four samples were taken from each horizon to eliminate individual variations. Whenever pebbles were found in the sample the method of calculating the volume weight was modified, as described elsewhere (3). No samples for volume weight could be obtained by this method from horizon A_0, the so-called leaf mold, which is shallow and does not lend itself to sampling with the tube. All other horizons were sampled 2 to 3 cm. away from the upper and lower border lines of the respective horizon to insure a representative sample.

Samples of soil for physical and chemical analyses were taken in the following manner: the spade was forced into the end wall of the soil at the border line of each horizon, the upper 2 to 3 cm. of soil removed,[4] and as much soil as neces-

[4] In the case of the leaf mold (A) horizon the undecomposed leaves and twigs were removed before sampling.

sary taken to a depth 2 to 3 cm. above the surface of the spade. This insured a representative sample. The soil material was placed into suitable bags, labelled, brought to the laboratory, dried at room temperature, sieved through a 2-mm. sieve, and stored in glass jars ready for physical and chemical analyses. This phase of the work is now in progress, and the results will be published as they accumulate.

SUMMARY

1. A brief discussion is presented on the advantages of studying soils in their profile make-up over the old method of studying the soil material sampled at arbitrary depths.

2. The methods used in the study of the soil profile are outlined somewhat in detail and discussed.

3. The following subjects are taken up: (*a*) Choice of locality for making the soil cut; (*b*) digging the soil cut; and (*c*) examination of the morphological features.

4. The methods of making the observations on the morphological features and of recording them are discussed under the following headings: Constitution, Habitus of the profile, Depth of profile and thickness of respective horizons, Texture of soil material, Color of soil, Structure, Concretions and foreign intrusions, and Miscellaneous observations such as depth of root penetration and distribution of organic matter.

5. Methods are presented and illustrated (*a*) on sampling the soil for volume weight (*b*) on sampling the individual horizons for soil material to be used in studies on the physical and chemical aspects of the soil.

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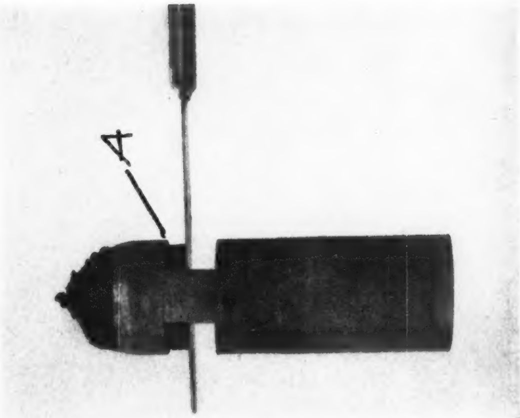
PLATE 1

Tube Used for Sampling Soils for Volume-weight Determinations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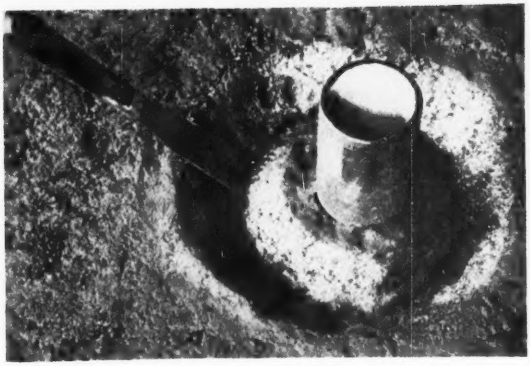

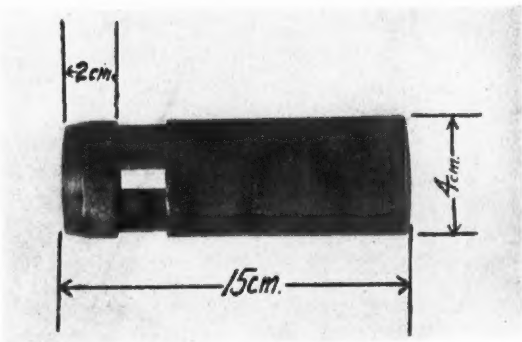
2cm
4cm.
15cm.

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SUBJECT INDEX

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